MICROBIAL PROCESSES IN EVERGLADES WETLAND SOILS

By

ALAN L. WRIGHT

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2001

ACKNOWLEDGMENTS

This research was supported in part from funding by the South Florida Water Management District, the U. S. Environmental Protection Agency, and the National Science Foundation. I would like to express appreciation to my major professor, Dr. K. R. Reddy, and all committee members (Dr. B. L. McNeal, Dr. D. A. Graetz, Dr. J. J. Delfino, and Dr. D. P. Chynoweth) for comments and suggestions regarding research, manuscripts, and the preparation of the dissertation. In addition, laboratory personnel who assisted with research problems and analysis include Y. Wang, G. Wilson, and R. Elliott. Other faculty members who contributed to research included Dr. W.F. DeBusk and Dr. J. R. White. I would also like to express appreciation to Dr. S. Newman and Dr. P. V. McCormick of the South Florida Water Management District for their assistance with research.

TABLE OF CONTENTS

page

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MICROBIAL PROCESSES IN EVERGLADES WETLAND SOILS

By

Alan L. Wright

December, 2001

Chairman: Dr. K. R. Reddy
Major Department: Soil and Water Science

Phosphorus loading to the Everglades wetland ecosystems has been implicated in altering natural vegetation patterns, increasing soil accretion rates, and influencing various soil and microbial biogeochemical indicators. The influence of nutrient loading, particularly phosphorus (P), on the activities of various extracellular enzymes, heterotrophic microbial activities, and other microbial processes along several P gradients located in four hydrologic units of the Florida Everglades was the focus of this study.

The alkaline phosphatase activity (APA) was inversely related to many soil P parameters and was sensitive to changes in P concentrations; thus APA was an excellent indicator of P enrichment. Phosphorus loading also enhanced heterotrophic microbial activities such as carbon dioxide and methane production rates. Heterotrophic microbial activities appeared to be controlled in part by microbial biomass in addition to nutrients, such as organic carbon and ammonium. The most sensitive indicators of P enrichment were soil P-related parameters including total P, total inorganic P, labile P, and potentially mineralizable P. Other biogeochemical indicators such as

microbial biomass were related to inorganic nutrients while heterotrophic microbial activities were related to microbial biomass in addition to inorganic nutrients.

Addition of various inorganic electron acceptors to soil showed that aerobic respiration was the dominant pathway of organic matter degradation, followed by anaerobic processes including denitrification and sulfate reduction. Methanogenesis contributed only a small portion of overall organic matter degradation. Addition of various electron donors to soil enhanced heterotrophic microbial activity in both P-impacted and unimpacted areas, suggesting that microbial activity was limited by labile organic C. Potential impacts of P loading on soil biogeochemical indicators were dependent on background soil total P levels of respective hydrologic units. Hydrologic units which exhibited low background P levels were more sensitive to P loading.

Enhanced heterotrophic microbial activity due to P loading has the potential for causing rapid turnover of organic matter, resulting in increased nutrient regeneration from soil and increased nutrient concentrations in the water column, thus adversely affecting the Everglades wetland ecosystem.

CHAPTER 1
INTRODUCTION

Overview

Organic matter in wetlands is important because of its influence on nutrient cycling, plant productivity, and water quality. Nutrient release during organic matter degradation or from external nutrient loading may result in the accumulation of nutrients in the water column, resulting in potential adverse effects on water quality and alterations in the biological components of wetlands (Fenchel and Jorgensen, 1977; Chrost, 1991; Reddy and D'Angelo, 1994). The understanding of pathways of organic matter degradation and nutrient cycling in wetland soils is important to determine the potential impacts of nutrient enrichment on various microbial processes which regulate organic matter degradation.

Organic matter degradation and nutrient cycling in wetland soils are dependent on factors such as hydrologic conditions, chemical characteristics of organic matter that affect substrate quality, availability of electron acceptors, and availability of growth-limiting nutrients (Webster and Benfield, 1986; McKinley and Vestal, 1992; Happell and Chanton, 1993; Amador and Jones, 1995; DeBusk and Reddy, 1998; Wright and Reddy, 2001b). The majority of organic matter in aquatic sediments is composed of highly complex, high-molecular-weight compounds (Chrost, 1991). Only small molecular-weight compounds dissolved in soil solution can pass through microbial cell membranes from the soil environment. Thus, only a small percentage of the dissolved organic carbon

(DOC) pool is available for direct microbial uptake (Chrost, 1991). To utilize DOC, heterotrophic microorganisms must hydrolyze large compounds through the production of extracellular enzymes. This hydrolysis is generally considered the rate-limiting step in organic matter decomposition in aquatic environments (Chrost, 1991).

Many extracellular enzymes play important roles in various pathways of organic matter degradation and in carbon (C), nitrogen (N), and sulfur (S) cycling in wetland soils (Harrison and Mann, 1975; Chrost, 1991). Following enzymatic hydrolysis of large organic compounds, microorganisms utilize these newly formed organic compounds as carbon or energy sources (Wetzel, 1991; DeBusk et al., 2001b). Through the process of enzymatic breakdown of organic matter, DOC and nutrients, such as ammonium (NH_4) and phosphate (PO_4), are released into the soil environment.

Wetland soils differ from upland soils in that they are subjected to periodic flooding and drying; thus, they support both aerobic and anaerobic microbial communities (D'Angelo and Reddy, 1999; DeBusk et al., 2001b). Organic matter degradation in wetland soils is highly dependent on the availability of electron acceptors, and a sequential reduction of electron acceptors occurs in flooded soils. A gradient of redox potentials in wetland soils favors the reduction of electron acceptors based on thermodynamically determined standard redox potentials (Ponnamperuma, 1972; Reddy and D'Angelo, 1994). The preferential use of electron acceptors by soil microorganisms occurs in the following order of preference: oxygen (O_2), nitrate (NO_3), sulfate (SO_4), and carbonates. In wetland soils, O_2 is present along a thin oxidized layer at the soil-water interface and along oxidized root channels. These oxidized layers have formed due to O_2 diffusion from the atmosphere to the soil surface or due to O_2 transfer through plants and

roots to soil. Concentrations of thermodynamically favored electron acceptors decrease with increasing depth, corresponding to increases in the dominance of anaerobic microorganisms. Oxygen is the most preferred electron acceptor by soil microorganisms, it is rapidly utilized by aerobic microbial communities and rarely detected beyond the soil surface and root channels (Reddy and D'Angelo, 1994). Aerobic microorganisms utilize O_2 in respiratory pathways, while anaerobic microorganisms function using alternate electron acceptors including NO_3 and SO_4.

Generally, aerobic microorganisms are more efficient in processes such as organic matter degradation and microbial respiration (D'Angelo and Reddy, 1999; DeBusk and Reddy, 1998; Wright and Reddy, 2001b). In fact, aerobic respiration rates are often several orders of magnitude greater than rates under anaerobic conditions (DeBusk and Reddy, 1998; Wright and Reddy, 2001b). Highest rates of heterotrophic microbial activity are often observed near the soil surface or in drier soil with O_2 used as an electron acceptor, while lowest rates are observed in flooded soils under SO_4 reduction and methanogenesis (Ponnamperuma, 1972; Howarth and Teal, 1979; D'Angelo and Reddy, 1999). Soils at greater depths are often older and thus more resistant to degradation than surface soils (Clymo, 1983). Freshwater wetlands are also generally limited by electron acceptors rather than by available C (Westermann, 1993), due to rapid depletion of O_2 in flooded surface soils.

Typical limiting nutrients in wetland soils include inorganic N and P and, due to the general abundance of organic C in wetland soils, these nutrients often limit organic matter degradation (Westermann, 1993). Microorganisms and vegetation compete for available nutrients in oligotrophic wetlands with limited nutrient input (Lodge et al.,

1994). Addition of limiting nutrients to oligotrophic wetlands results in changes of vegetation and of microbial community structure and function, often resulting in assimilation of available nutrients into microbial biomass (Chrost, 1991; Wright and Reddy, 2001a,b). Nutrients are obtained by soil microorganisms from external loading or from regeneration during organic matter degradation (Godshalk and Wetzel, 1978; Reddy and D'Angelo, 1994). Nutrient additions to wetland soils, particularly NO_3 and SO_4, tend to inhibit methanogenesis because methanogens are out-competed for substrates by aerobic, denitrifying, or SO_4-reducing microorganisms (Winfrey and Zeikus, 1977). This inhibition of methanogenesis has been suggested to be due to competition for substrates (Winfrey and Zeikus, 1977) or to thermodynamic constraints (Zehnder, 1978).

The DOC pool of wetland soils is relatively stable both in size and quality. However, not all of the DOC pool is available to soil microorganisms. Decomposition of organic matter in wetland soils often occurs in two phases: the initial short-term rapid breakdown of labile portions of the DOC pool, followed by a longer-term steady breakdown of the more recalcitrant portion of the DOC pool (Chrost, 1991). This second phase is often dependent on microbial biomass and the production of extracellular enzymes in addition to the availability of nutrients.

The microbial biomass of soils is a strong regulating component of various transformations and processes including enzyme production, heterotrophic microbial activity, and organic matter degradation (DeBusk et al., 2001b). Soil microorganisms derive energy and carbon from the breakdown of detrital and soil organic matter, which facilitates nutrient cycling in soils (Wetzel, 1991). Microbial biomass and organic matter degradation may be limited by available nutrients if sufficient supply is not met from

nutrient regeneration or from soil solution (Godshalk and Wetzel, 1978). This may result in immobilization of nutrients into soil microbial biomass (Melillo et al., 1984). Microbial biomass is often increased under aerobic conditions compared to the anaerobic conditions of flooded soils (D'Angelo and Reddy, 1999). Measurement of microbial biomass and related heterotrophic microbial activities provides an indication of wetland function and also of organic matter and nutrient cycling.

Site Description

The Florida Everglades consists of a series of wetland ecosystems extending from Lake Okeechobee south to Florida Bay (Fig. 1.1). The Everglades historically developed under nutrient-limited conditions and underwent periodic flooding and drying. Water inputs in the Everglades were historically from overflow of Lake Okeechobee or from rainfall (Davis, 1943). Major vegetational communities included *Cladium* (sawgrass) intermingled with open sloughs, wet prairies, and tree islands. Developments in past decades in the Everglades included the construction of a series of canals and impoundments that have altered water movement through the Everglades. In addition, drainage of the northern Everglades and subsequent agricultural practices have led to external nutrient loading from the Everglades Agricultural Area (EAA) into various hydrologic units of the Everglades. This has resulted in increased soil nutrient concentrations, and particularly P (McCormick et al., 2000). In addition, vegetational changes due to nutrient loading and to altered hydrologic conditions have resulted in intrusions of *Typha* (cattail) into various Everglades wetlands (McCormick et al., 2000; DeBusk et al., 2001a).

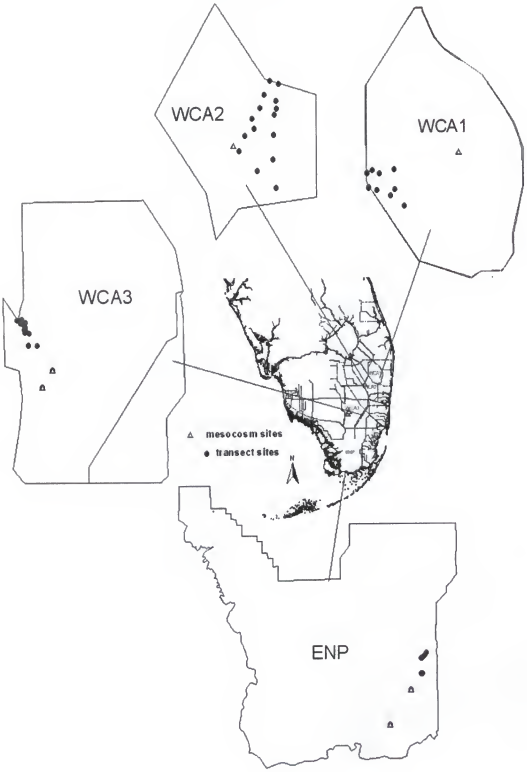

Figure 1.1. Locations of experimental sites along nutrient gradients in Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough of Everglades National Park (ENP).

The research sites for this study were located in four hydrologic units of the Everglades: Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough (TS) of Everglades National Park (ENP) (Fig. 1.1). All four hydrologic units have been impacted to some degree by external nutrient loading; however, northern hydrologic units have experienced higher P loadings (McCormick et al., 2000). Nutrient-impacted areas in the various hydrologic units are primarily located near to or adjacent to water inflow points or canals. Gradients in plant primary productivity, corresponding to soil P concentrations, are evident along P-enrichment gradients in the various hydrologic units from regions near inflow waters extending into the interior of the hydrologic units. Soils in P-impacted areas consist of thick layers of partially-decomposed plant material (detritus) overlaying a more consolidated, decomposed soil. In P-unimpacted areas located in the interior of hydrologic units, detritus is present in vegetated areas. However, in unvegetated areas, floc accumulations are present above the soil surface and in the water column, consisting of calcareous benthic periphyton and floating algal mats. Beneath the detritus and floc is the more consolidated, decomposed soil.

Water Conservation Area-2a was the primary hydrologic unit studied. It is located in the northern part of the Everglades wetland ecosystem, south of the EAA and WCA-1. It is a freshwater wetland traditionally occupied by *Cladium* communities interspersed with open water sloughs. However, over the past several decades, external inputs of nutrients (particularly P) have been implicated in causing a vegetation community shift from *Cladium* and calcareous periphyton to a *Typha* dominated system. Traditionally, water generally flowed in a north to south direction. In past decades, however, a series of canals, dikes, and impoundments have been implemented for flood

control and municipal development. These changes have altered the natural flow of water through WCA-2a.

The WCA-1 is a national wildlife refuge encompassing 59,000 ha of the northern Everglades ecosystem. Rainfall is the primary water input while other water inputs include P-laden runoff from the EAA. Most of the increased soil TP levels have been observed in areas adjacent to canals or levees (McCormick et al., 2000). *Typha* predominates in P-impacted areas while *Cladium*, open sloughs, and tree islands are common in unimpacted areas in the interior of WCA-1.

The WCA-3a receives drainage water from northern areas, particularly WCA-2a, but rainfall contributes approximately 42% of its annual water budget (Reddy et al., 1998). In WCA-3a, tree islands and wet prairies primarily comprise the vegetation community structure. Sites near water inflow structures exhibit enhanced soil P concentrations, which then decrease in the interior of WCA-3a.

Taylor Slough is located south of WCA-3a and serves as a bridge from the Everglades National Park south to Florida Bay, and TS receives discharge water from WCA-3a. The soils of TS differ from those of other hydrologic units as the wet prairies in TS formed on marl sediments under P-limited conditions. The P-impacted areas of TS are not as well defined as in other hydrologic units, but soil TP levels are highest near water inflow points.

Objectives

The overall objective of the study was to determine the impacts of P loading on microbial processes in Everglades wetland soils. A diagram showing the research topics is presented in Fig. 1.2. Specific objectives were to:

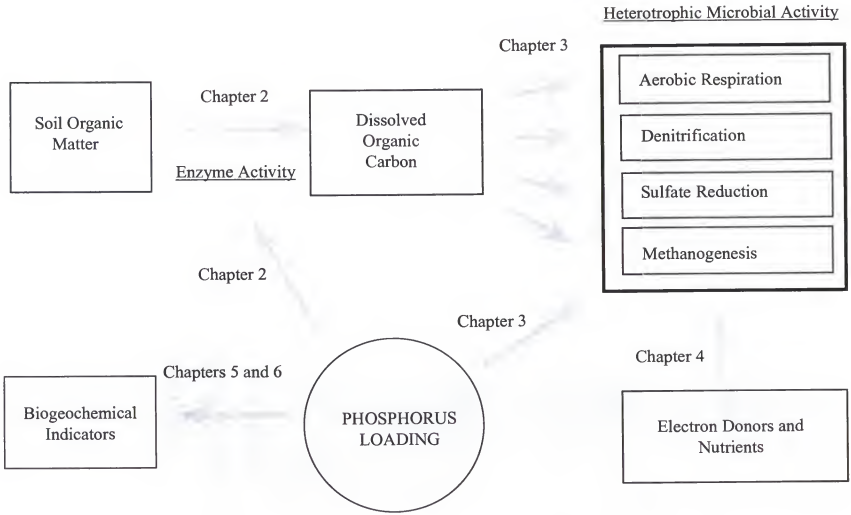

Figure 1.2. Microbial processes investigated in Everglades wetland soils.

1) Determine the impacts of nutrient loading on extracellular enzyme activity in detritus and soil. Develop relationships between extracellular enzyme activities and various soil biogeochemical indicators. It was hypothesized that nutrient loading would enhance extracellular enzyme activities.
2) Determine the impacts of nutrient loading, electron acceptors, electron donors, and inorganic nutrients on heterotrophic microbial activities in various hydrologic units of the Everglades. Relationships between various microbial processes and soil biogeochemical indicators will also be developed. It was hypothesized that nutrient loading would enhance heterotrophic microbial activity. Additions of electron acceptors and donors would also likely enhance microbial activity.
3) Develop soil biogeochemical indicators of P enrichment in various hydrologic units of the Everglades. Determine which indicators are sensitive to P loading. Investigate differences of biogeochemical indicators between various hydrologic units and in their response to nutrient loading. It was hypothesized that P-related parameters would be sensitive to P loading and that impacts of P loading on biogeochemical indicators would be similar among the four hydrologic units.

Experimental data were gathered from both field and laboratory experiments. The impacts of nutrient loading on extracellular enzymes activities are presented in Chapter 2. Impacts of added electron acceptors on heterotrophic microbial activities along a P-enrichment gradient are presented in Chapter 3. The impacts of added electron donors and inorganic nutrients on heterotrophic microbial activities are presented in Chapter 4. The changes in soil biogeochemical indicators along various nutrient-enrichment

gradients of four hydrologic units of the Everglades are the focus of Chapter 5 while the influence of experimental P dosing on various soil biogeochemical indicators is presented in Chapter 6. Conclusions of experiments and summaries of results are presented in Chapter 7.

The completion of these objectives should provide insights on the relationships between extracellular enzyme activity, heterotrophic microbial activity, soil biogeochemical indicators, and organic matter degradation of Everglades soils. In addition, sensitive indicators of P enrichment for various hydrologic units of the Everglades should be identified.

CHAPTER 2
PHOSPHORUS LOADING EFFECTS ON EXTRACELLULAR ENZYME ACTIVITY IN EVERGLADES WETLAND SOILS

Introduction

Wetland soils support a large diversity of microbial communities that play important roles in the decomposition of organic matter, nutrient cycling, and abating the toxic levels of contaminants. The majority of organic matter in wetlands and aquatic systems is composed of high molecular weight, polymeric compounds, of which only a small portion is readily available to microbial communities (Benner et al., 1984; Chrost, 1991). Complex structural compounds must be first hydrolyzed through the activity of extracellular enzymes into low molecular-weight compounds (Chrost, 1991; Sinsabaugh et al., 1991). These low molecular-weight compounds can be directly transferred to microbial cells, oxidized, and used as energy sources (Chrost, 1991). Nutrient loading can potentially alter the activity of extracellular enzymes, which is critical for the first step of the degradation of soil organic matter and detrital plant tissue (Halemejko and Chrost, 1984; Sinsabaugh et al., 1992).

Several enzymes are known to be involved in the cycling of nutrients, and can be used as potential indicators of soil nutrient cycling processes (McLatchey and Reddy, 1998). In P-limited wetlands or in aquatic ecosystems, alkaline phosphatase activity (APA) plays an important role in the regeneration of inorganic P from organic P through its catalysis of the breakdown of organic P esters to inorganic P (Chrost, 1991). Since up

to 90% of organic P may be in monoester form (Condron et al., 1985), the role of APA in P regeneration from soils is important. Alkaline phosphatase activity is often repressed by high dissolved reactive P concentrations in a process referred to as feedback inhibition (Cembella et al., 1984; Chrost, 1991). Similarly, arylsulfatase and protease enzymes function in nutrient cycling by regenerating inorganic SO_4 and NH_4 from organic matter. Arylsulfatase catalyzes the hydrolysis of SO_4 esters resulting in the release of inorganic SO_4 (Tabatabai and Bremner, 1970); thus, it is likely important to S cycling processes in wetland soils. Protease enzymes are important for the wetland N cycle and function in the breakdown of proteins, resulting in the release of NH_4 (Ladd and Butler, 1972). Glucosidase catalyzes the hydrolysis of glycosides, resulting in the release of a monosaccharide (Eivazi and Tabatabai, 1988). Phenol oxidase is important in the breakdown of lignin-containing compounds and is dependent on the availability of O_2 (Pulford and Tabatabai, 1988; Pind et al., 1994; McLatchey and Reddy, 1998). Cellulose and lignin degrading enzyme activities have also been correlated with degradation rates of detritus (Sinsabaugh et al., 1994; McLatchey and Reddy, 1998).

Microbiological properties, including enzyme activities, can be potentially useful as indicators of soil and water quality. Many of these enzymes are affected by nutrient loading, as bioavailable nutrients can potentially decrease their activity (Chrost, 1991; Wetzel, 1991). Measurement of extracellular enzyme activities may therefore be useful for predicting the impacts of P loading (Gage and Gorham, 1985; Wetzel, 1991; Whitton 1991; Newman and Reddy, 1993), since enzyme activity is important to organic matter degradation, nutrient regeneration, and various elemental cycles.

We measured the activity of various extracellular enzymes in detritus and soil samples collected along a P gradient located in Water Conservation Area-2a (WCA-2a) of the Florida Everglades. The extracellular enzymes assayed included alkaline phosphatase (APA), arylsulfatase, glucosidase, protease, and phenol oxidase. The objectives of the study were to determine: (1) the influence of P loading on activities of various extracellular enzymes in detritus and soil along a P gradient; (2) the relationship between extracellular enzyme activities and selected microbial and soil physico-chemical parameters; and (3) to determine whether these enzyme activities are useful as sensitive indicators of P loading.

Materials and Methods

Site Description

The study site is WCA-2a of the Florida Everglades. This 447 km^2 impounded wetland has received P-laden drainage waters for the past several decades from the adjacent Everglades Agricultural Area (EAA). Inflow of these waters has been implicated in contributing to increased P concentrations of the soil and water column (Reddy et al., 1993; DeBusk et al., 1994). Historically, this system was P limited and contained a mixture of sawgrass (*Cladium* sp.) and slough communities (Davis, 1991). The addition of P-laden waters and altered hydrology have been implicated as key factors in a vegetation shift from the indigenous sawgrass/slough communities to cattail (*Typha* sp.) dominated communities, resulting in increased peat accumulation and additional soil and water alterations (Davis 1991; Craft and Richardson, 1993; Reddy et al., 1993). Soils near the inflow have the highest P concentrations, and P concentrations decrease with increasing distance from the inflow. Corresponding to changes in soil P concentrations, a

gradient in vegetative community type exists as well. *Typha* is the dominant vegetative type in areas impacted by P, while *Cladium* is prominent in P-unimpacted areas (Davis, 1991; DeBusk et al., 1994).

Detritus and Soil Sampling and Characterization

Detritus and soil samples were collected from eight stations along a P gradient, 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4, and 10.1 km south of the primary point of water inflow (S10-C) in WCA-2a of the Everglades (Table 2.1). Sampling sites encompassed different levels of P enrichment (approximately 2000-400 mg P kg $soil^{-1}$) and different vegetative community types (*Typha*, mixed area, *Cladium*/sloughs). Samples were collected in February, May, and August 1996, and in March 1997 to determine seasonal fluctuations of measured parameters. Samples consisted of recently-deposited, distinguishable surface plant detritus and two soil depth intervals. Detritus was collected at each of the sampling stations from above the cored soil. Soil cores were obtained by driving an aluminum corer (i.d.=14.6 cm) to a depth of approximately 40 cm. At each sampling station, four soil cores were obtained approximately 1-2 m apart.

The only exception was that only one core was taken during the May sampling period. This sampling period was utilized only for enzyme activity measurements and not for detailed soil characterization; thus, only 1 core was taken. Soil cores were sectioned into 0-10 and 10-30 cm layers, and respective layers of all four cores were combined into one bulk sample and homogenized for use in experiments. Since we were interested in sampling along the P gradient, no attempt was made to determine spatial variability at each of the eight stations. Samples were placed in plastic bags and stored

Table 2.1. Station locations along the nutrient gradient in Water Conservation Area-2a.

Station	Distance from Inflow	Latitude N.		Longitude W.	
	(km)	(deg)	(min)	(deg)	(min)
1	1.40	26	21.53	80	21.20
2	2.27	26	21.05	80	21.21
3	3.25	26	20.53	80	21.27
4	4.19	26	20.02	80	21.37
5	5.12	26	19.52	80	21.39
6	6.98	26	18.51	80	21.46
7	8.41	26	17.73	80	21.42
8	10.13	26	16.81	80	21.48

on ice for transport back to the laboratory. Subsequently, all samples were stored in a refrigerator at 4°C until analysis.

Various soil and microbial parameters were determined on well-mixed subsamples and are presented in Tables 2.2 and 2.3. Soil and microbial parameters were expressed on a dry weight basis. Total C and N on detritus and soil were determined on oven-dried (70°C) ground samples using a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Microbial biomass C was determined using a fumigation-extraction procedure (Vance et al., 1987), with 0.5 *M* K_2SO_4 extracts being analyzed using a Dohrman total C analyzer (Rosemount Analytical, Santa Clara, CA). Extractable NH_4-N was determined by the method of Mulvaney (1996) using an automated colorimetric procedure (U. S. EPA, 1993b). Sulfate concentrations were determined by ion chromatography (U. S. EPA, 1993a). Soil total P was determined by nitric-perchloric acid digestion (Kuo, 1996) and analyzed using an automated ascorbic acid colorimetric procedure (U. S. EPA, 1993c). Microbial biomass P was determined by a fumigation-extraction method (Hedley and Stewart, 1982). The $NaHCO_3$-P*i* fraction represented the non-fumigated 0.5 *M* $NaHCO_3$-extractable inorganic P.

Sample Preparation and Enzyme Assays

In the laboratory, subsamples were taken from bulk samples and were further homogenized. Large roots or shells were removed, as they were deemed inappropriate for analysis. Approximately 0.5-0.75 g wet samples were added to polypropylene centrifuge tubes for analysis of most enzyme activities. Assays were performed using either three or four replications and appropriate controls to account for non-enzymatic color development. Colorimetric methods used in the enzyme assays were as follows:

Table 2.2. Soil physico-chemical properties in Water Conservation Area-2a averaged from the February and August 1996 and March 1997 sampling periods (standard error).

Depth	Sites	Bulk Density	Total C	Total N	Microbial Biomass C	Ammonium	Sulfate
		$g\ cm^{-3}$	$g\ C\ kg^{-1}$	$g\ N\ kg^{-1}$	$g\ C\ kg^{-1}$	$mg\ N\ L^{-1}$	$mg\ S\ L^{-1}$
Detritus	P-Impacted	n.d.	442 (6)	25 (1)	17 (1)	0.43 (0.1)	12.3 (3.5)
	Transitional	n.d.	427 (10)	22 (1)	13 (2)	0.61 (0.1)	4.2 (0.8)
	Unimpacted	n.d.	412 (7)	24 (2)	8 (2)	0.39 (0.2)	8.0 (2.0)
0-10 cm	P-Impacted	0.06 (0)	432 (11)	28 (1)	5 (1)	2.21 (0.8)	6.4 (1.8)
	Transitional	0.07 (0)	436 (10)	29 (1)	4 (1)	1.30 (0.2)	2.8 (0.6)
	Unimpacted	0.06 (0)	443 (10)	29 (1)	4 (1)	0.86 (0.1)	2.9 (0.4)
10-30 cm	P-Impacted	0.15 (.02)	461 (7)	32 (1)	2 (0)	2.49 (0.7)	4.8 (0.8)
	Transitional	0.14 (.02)	430 (23)	28 (2)	2 (0)	1.82 (0.3)	1.8 (0.4)
	Unimpacted	0.12 (.02)	476 (9)	29 (2)	1 (0)	1.34 (0.4)	1.3 (0.2)

Table 2.3. Selected forms of P in detritus and soils in Water Conservation Area-2a averaged from the February and August 1996 and March 1997 sampling periods (standard error).

Depth	Sites	Total P	$NaHCO_3$-P*i*	Microbial Biomass P
		----------mg P kg^{-1}----------		
Detritus	P-Impacted	1575 (62)	96 (25)	328 (31)
	Transitional	1140 (131)	50 (17)	296 (37)
	Unimpacted	486 (102)	8 (2)	150 (52)
0-10 cm	P-Impacted	1375 (58)	23 (3)	110 (13)
	Transitional	865 (67)	16 (3)	86 (8)
	Unimpacted	484 (56)	7 (1)	76 (14)
10-30 cm	P-Impacted	435 (39)	9 (1)	39 (3)
	Transitional	274 (18)	5 (1)	38 (2)
	Unimpacted	237 (19)	5 (1)	28 (4)

alkaline phosphatase (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977), arylsulfatase, glucosidase (Eivazi and Tabatabai, 1988), protease (Ladd and Butler, 1972), and phenol oxidase (Pind et al., 1994). All enzyme activities were expressed on a dry weight basis.

Alkaline phosphatase, arylsulfatase, and glucosidase were analyzed using similar substrates and methodology. The base substrate utilized was *p*-nitrophenol bound with phosphate, SO_4, or glucose (Sigma Chemical Co, St. Louis, MO). The artificial substrate (1 mL, 0.05 *M*), toluene (to inhibit microbial growth during incubation), a pH buffer (pH = 11 for APA, 5.8 for arylsulfatase, 6.0 for glucosidase), and 0.50-0.75 g wet samples were incubated in closed polypropylene centrifuge tubes at 37°C for 1 hr. After incubation, enzyme activity was stopped by addition of 4 mL of 0.5 *M* NaOH (APA and arylsulfatase) or 4 mL of 0.5 *M* THAM (glucosidase), with 1 mL of 0.5 *M* $CaCl_2$. The mixture then was filtered and the extract analyzed using an UV-VIS spectrophotometer (Shimadzu Model UV-160) at 420 ηm. Absorbance of filtrates was compared to *p*-nitrophenol standards. To account for non-enzymatic substrate hydrolysis, values for controls were subtracted from sample replicates.

Protease activity was determined using 0.5-0.75 g wet soil, 2.5 mL of casein (10 mg mL^{-1}) in 0.1 *M* THAM buffer at pH = 8.1. This mixture was incubated at 37°C for 1 hr. Enzyme activity was stopped by addition of 1 mL of 17.5% trichloroacetic acid. After centrifugation, 2 mL of supernatant was mixed with 3 mL of 2.8 *N* Na_2CO_3 and 1 mL of Folin reagent. The absorbance of the resulting solution was measured at 700 ηm and compared to tyrosine standards.

Phenol oxidase activity was measured using a slurry of 0.1-0.3 g wet samples and 5 mL water mixed with 10 m*M* of L-DOPA (dihydroxyphenylalanine) solution. Incubation time varied between 1 and 3 min, and the difference in absorbance between incubation times was used to calculate phenol oxidase activity. After incubation, mixtures were filtered and analyzed at 400 ηm. Calculation of the quantity of diqc (dihydroindole-quinone-carboxylate) released was based on a 1 cm light path and Beer's Law using a molar absorbance coefficient of $3.7*10^4$ (Mason, 1948).

Data Analysis

Enzyme activities were compared to determine differences in soil depth, sampling station, and season using ANOVA and Fisher's LSD at $P<0.05$ (CoStat, Minneapolis, MN). Linear correlation coefficients were also determined between enzyme activity and various soil physico-chemical properties. In order to eliminate seasonal variability, APA was expressed as a percentage of maximum activity for a given season and soil depth. To determine field variability for ANOVA, the eight sampling stations along the P gradient were grouped into three separate units based on vegetative type and total soil P concentrations. The three groupings included field stations 1, 2, and 3 (*Typha* growing in P-impacted areas), stations 4, 5, and 6 (mixed vegetational areas in a transitional zone), and stations 7 and 8 (*Cladium* growing in P-unimpacted areas) for expression of arylsulfatase, glucosidase, protease, and phenol oxidase activities. Statistical differences from ANOVA at $P<0.05$ were compared between the impacted, transitional, and unimpacted areas.

Results and Discussion

Soil bulk density increased with soil depth but was not affected by P loading (Table 2.2), and soil pH generally ranged from 7-8 (data not shown). Total C and N were not affected by P loading or soil depth. Total P, $NaHCO_3$-P*i*, microbial biomass C, and microbial biomass P were highest in surface detritus and decreased both with soil depth and with distance along the P gradient (Tables 2.2 and 2.3).

Alkaline Phosphatase

For all sampling periods, APA was highest in the surface detrital layer and significantly decreased ($P<0.05$) with depth in the soil profile (Fig. 2.1). Similar decreases in phosphatase activity with depth have been observed for other aquatic systems (DeGobbis et al., 1984; Newman and Reddy, 1992). Alkaline phosphatase activity was approximately 10-15 fold higher in detritus than in underlying soil at unimpacted sites. The detritus consisted of partially-degraded, recently deposited plant material, either from *Typha* or *Cladium*. Substrate quality and perhaps aerobic conditions in surface detritus make this material more utilizable for microorganisms than underlying soils (DeBusk, 1996); thus, APA would be higher in detritus. Therefore, a decrease in APA with depth would correspond to a decrease in substrate quality with depth.

Since APA varied seasonally, activities were normalized to maximum activity measured during that particular season and expressed as a percentage of the maximum rate for a given soil depth and season (Table 2.4). Data for all sampling periods were combined for each depth to provide an indication of P loading effects for the various seasons (Fig. 2.2). Alkaline phosphatase activity in the detritus and 0 - 10 cm soil layer significantly increased ($P<0.05$) with increasing distance from the inflow and was highest

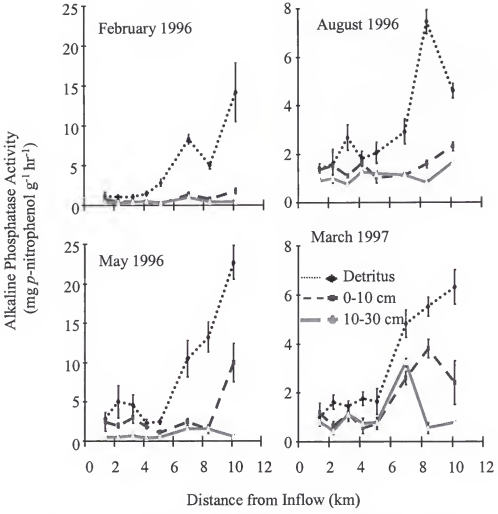

Figure 2.1. Alkaline phosphatase activity (mg *p*-nitrophenol g soil^{-1} hr^{-1}) in detritus and at two soil depths along the nutrient gradient in WCA-2a for four sampling times. Error bars represent standard error.

Table 2.4. Alkaline phosphatase activity expressed as a percentage of maximum [(APA/APA *maximum*)*100] for each soil depth and sampling period. The stations and (standard error) are presented.

Sampling Period	Station	Detritus	Station	0-10 cm	Station	10-30 cm
Feb-96	8	14.1 (3.6)	8	1.8 (0.3)	6	0.9 (0.1)
May-96	8	22.7 (2.1)	8	9.9 (2.4)	6	1.6 (0.1)
Aug-96	7	7.4 (0.5)	8	2.3 (0.2)	8	1.6 (0.1)
Mar-97	8	6.3 (0.7)	7	3.8 (0.4)	6	3.2 (0.2)

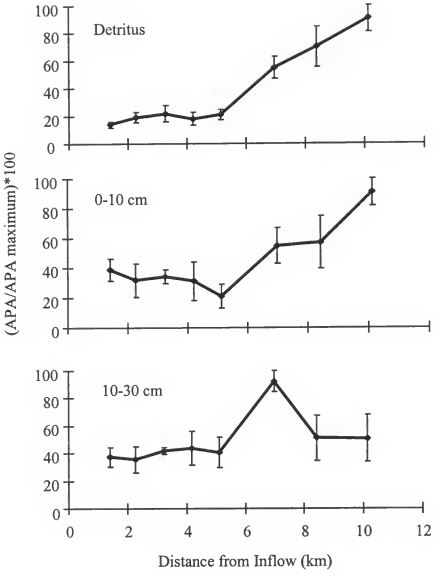

Figure 2.2. Alkaline phosphatase activity in detritus and at two soil depths along the nutrient gradient in WCA-2a expressed as a percentage of maximum APA for each sampling period. Data for each depth represent an average of four sampling times. Error bars represent standard error.

at sites >5 km from the inflow. The APA levels were generally constant up to 5 km from the inflow but then increased along the P gradient as P levels in the detritus and 0-10 cm soil depth decreased. However, APA activity remained fairly constant in the 10-30 cm soil depth along the gradient. The significant response of APA to P loading in detritus and 0-10 cm soil may be the result of increased microbial activity in surface soils due to the greater availability of labile organic C than in deeper soils. The response of the microbial community to P loading would be more dynamic and sensitive in detritus and surface soil than in deeper soil, where lower C bioavailability rather than P concentrations may regulate microbial activity and APA. At lower depths much of the organic matter is well decomposed, resulting in high lignin/(lignin+cellulose) ratios and low microbial activity (DeBusk and Reddy, 1998). This results in minimal demand for bioavailable P. Such conditions resulted in very little or no trend in APA along the P gradient for 10-30 cm soil, even though there existed a wide range in soil P concentrations from impacted to unimpacted sites (approximately 600 to 200 mg P kg $soil^{-1}$). In addition, the water table was below the soil surface for most sampling periods, likely exposing detritus and surface soil to aerobic conditions and increasing microbial activity. Lower water table depths increased the decomposition process (DeBusk, 1996) and placed higher demand on bioavailable P.

Alkaline phosphatase activity in detritus (February and August 1996, and March 1997 sampling periods) was significantly ($P<0.05$) negatively correlated with total P ($r = -0.75$), $NaHCO_3$-P*i* ($r = -0.51$), and microbial biomass P ($r = -0.41$). Inverse relationships between soil P parameters and APA in soil have often been observed (Cotner and Wetzel, 1991; Newman and Reddy, 1993). However, relationships between APA and soil P

parameters may be attributed to other factors. Alkaline phosphatase production may be regulated by the microbial internal P pool, which may not accurately reflect the P pool outside of microbial cells (Chrost, 1991). Mineral inorganic P additions also have been reported to have stimulatory, inhibitory, and no effect on phosphatase activity in soils (Speir and Ross, 1978).

A non-linear relationship between (APA/APA *maximum)* and $NaHCO_3$-P*i* was observed for detritus (Fig. 2.3). Alkaline phosphatase activity appeared to be stimulated when $NaHCO_3$-P*i* decreased below 40 μg P L^{-1}. For the 0-10 cm depth interval, APA was only significantly ($P<0.05$) correlated with soil total P ($r = -0.48$), although negative relationships with other soil P parameters were observed but were insignificant. Changes in APA along the gradient corresponded to changes in both soil P concentrations and vegetation type (*Typha* to *Cladium*). However, changes in APA along the P gradient were not due to differences in vegetation between the *Typha* and *Cladium* areas. In a separate study using constructed mesocosms (built on similar vegetation type in an unimpacted open slough) that received various rates of P loading over several years, similar responses of APA to P loading were observed (McCormick et al., 2000). Thus, differences in APA along the P gradient appeared to be due to P loading rather than to vegetation or substrate quality.

Alkaline phosphatase activities measured in our study were generally higher than those observed for other aquatic systems and much greater than for agricultural soils. For example, APA values of up to 38,000 mg *p*-nitrophenol kg^{-1} hr^{-1} were observed for stream sediment, 1700 mg *p*-nitrophenol kg^{-1} hr^{-1} for suspended particulates in stream, and 2600 mg *p*-nitrophenol kg^{-1} hr^{-1} for fine particulate organic matter (Saylor et al.,

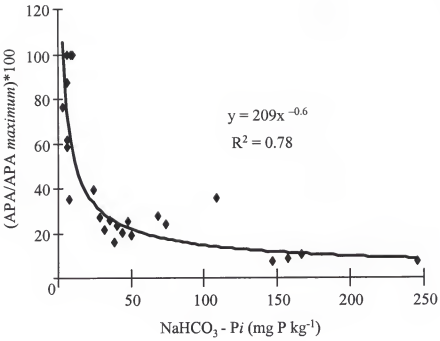

Figure 2.3. Alkaline phosphatase activity in detritus expressed as percentage of maximum plotted against bicarbonate extractable-P ($NaHCO_3$-P*i*). Data represent an average for the February and August 1996 and March 1997 sampling periods.

1979). Activities in shallow, nutrient-rich, freshwater surface sediments ranged from 1350 mg *p*-nitrophenol kg^{-1} hr^{-1} for unvegetated sediments to 1775 mg *p*-nitrophenol kg^{-1} hr^{-1} for vegetated sediments (Boon and Sorrell, 1991). In a reclaimed wetland, APA values ranged from 12-24 mg *p*-nitrophenol kg^{-1} hr^{-1} (McLatchey and Reddy, 1998). Rates of APA for many agricultural surface soils ranged from 20-235 mg *p*-nitrophenol kg^{-1} hr^{-1} (Juma and Tabatabai, 1978) but, for one soil, up to 5000 mg *p*-nitrophenol kg^{-1} hr^{-1} (Nannipieri et al., 1979). The high organic matter content in Everglades soil likely enhances microbial and enzymatic activity.

Arylsulfatase

Arylsulfatase activity in detritus and soil was not affected by P loading but significantly ($P<0.05$) decreased with depth (Fig. 2.4). However, arylsulfatase activity was not significantly related to floodwater SO_4 concentrations. Although arylsulfatase catalyzes the release of SO_4 from organic ester S, it has been suggested that arylsulfatase may also be produced for the breakdown of carbon-bonded organic S (Oshrain and Wiebe, 1979). Similar to APA, arylsulfatase activity was significantly ($P<0.05$) affected by soil depth, with higher activity observed in the detritus. Similar trends in arylsulfatase activity with depth have been reported for other systems (King and Klug, 1980). Detrital arylsulfatase activity was approximately two-fold higher than for soils from the 0-10 cm depth, and threefold higher than for soils from the 10-30 cm depth.

Arylsulfatase activity along the P gradient in WCA-2a was greater than most values reported in the literature for wetland soils and much greater than for agricultural soils, likely due to the higher organic matter and microbial biomass content of Everglades soils. Ranges of sulfatase activity in wetland peat were 550-850 $\mu g\ g^{-1}\ hr^{-1}$

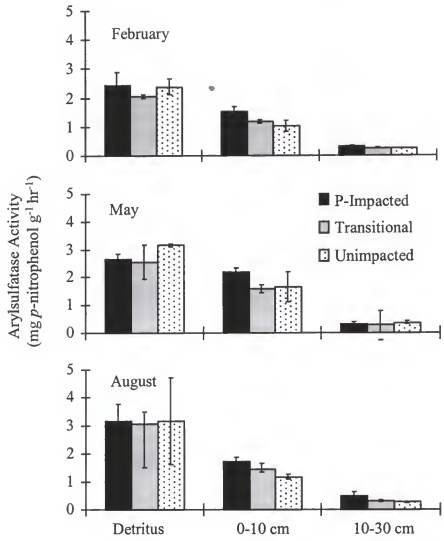

Figure 2.4. Arylsulfatase activity (mg *p*-nitrophenol g soil^{-1} hr^{-1}) in detritus and at two soil depths for the February, May, and August 1996 sampling periods in P-impacted, transitional, and unimpacted areas. Error bars represent standard error.

(Sarathchandra and Perrott, 1981; Press et al., 1985). Sulfatase activity in marine sediments was highest at the sediment surface (80 μg *p*-nitrophenol mL^{-1} hr^{-1}) and decreased with depth (10 μg *p*-nitrophenol g^{-1} hr^{-1}) (Oshrain and Wiebe, 1979). Arylsulfatase activity in freshwater lake sediments was highest at the surface (12 mg *p*-nitrophenol L^{-1} hr^{-1}) and decreased with depth (King and Klug, 1980), corresponding to decreases in O_2 content. Additions of inorganic phosphate, NO_3, and SO_4 have been found to depress sulfatase activity in peat (Press et al., 1985). Sulfatase activity in coastal sands was reported to be higher in vegetated areas (8.5 μg *p*-nitrophenol g^{-1} hr^{-1}) than in unvegetated areas (1.7 μg *p*-nitrophenol g^{-1} hr^{-1}) due to input of plant sulfatases (Skiba and Wainwright, 1983).

It has been suggested that sulfatases are not effective indicators of organic S mineralization, since there are many enzymes involved in these processes (Ladd and Jackson, 1982). Thus, these enzymes may not adequately reflect changes in soil SO_4 concentrations. In addition, sulfatases may be produced for use in supplying inorganic SO_4 for SO_4 reduction, which would further limit the role of sulfatase for estimating organic S mineralization (King and Klug, 1980).

Glucosidase

Phosphorus loading had no significant impacts on glucosidase activity of detritus and soil (Fig. 2.5). Glucosidase is an important extracellular enzyme involved in organic matter degradation, and it is responsible for conversion of cellobiose to glucose monomers (Eivazi and Tabatabai, 1988). Glucosidase activity in nutrient-enriched mesocosms was correlated with microbial production (Chrost and Rai, 1993), possibly due to increased organic matter inputs resulting from nutrient enrichment. In a related

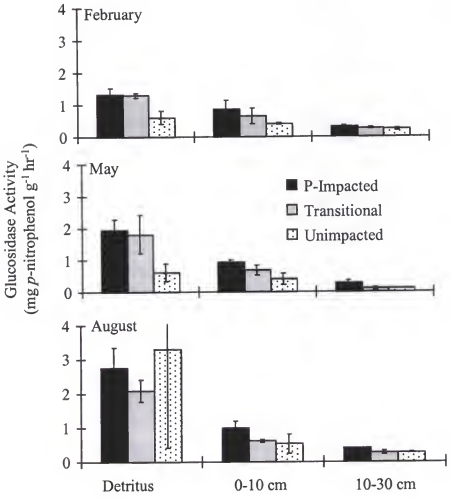

Figure 2.5. Glucosidase (mg *p*-nitrophenol g soil^{-1} hr^{-1}) activity in detritus and at two soil depths for the February, May, and August 1996 sampling periods in P-impacted, transitional, and unimpacted areas. Error bars represent standard error.

study of Everglades soil, microbial respiration rates were generally lowest at unimpacted sites compared to impacted and transitional sites (DeBusk and Reddy, 1998). Glucosidase activity was positively related to DOC in nutrient-enriched soil but not in nutrient-impoverished soil (Chrost and Rai, 1993). Thus, greater DOC content generally implies greater availability of substrates for microbial growth. Bacteria appeared to be limited by the supply of readily utilizable monomers under nutrient-enriched soil, but supply of substrates was not limited in nutrient-impoverished soil (Chrost and Rai, 1993). In a nutrient non-limited wetland soil, McLatchey and Reddy (1998) reported that glucosidase activity provided a good indication of C mineralization rates.

As with APA and arylsulfatase, glucosidase activity was significantly ($P<0.05$) higher in the detritus layer and decreased with depth. This again may be due to decreases in substrate quality with depth (DeBusk, 1996) or to O_2 decrease with depth. Though glucosidase activity was inversely related to redox potential, the flooding of a dry soil enhanced glucosidase activity (Pulford and Tabatabai, 1988). Glucosidase activity in a northern *Typha* marsh receiving farmland drainage ranged from 200 $\mu g\ g^{-1}\ hr^{-1}$ at the inflow site to 5000 $\mu g\ g^{-1}\ hr^{-1}$ at the outflow site, corresponding to increases in particle size (Jackson et al., 1995).

Protease

Protease activity was not affected by P loading as there were no statistically significant trends along the gradient in detritus or soil for either the May or August sampling periods (Fig. 2.6). Protease activity was highest ($P<0.05$) in the detritus layer and significantly decreased with depth, similar to other studies (Speir and Ross, 1978). Protease activity decreased with depth likely due to decreases in substrate quality,

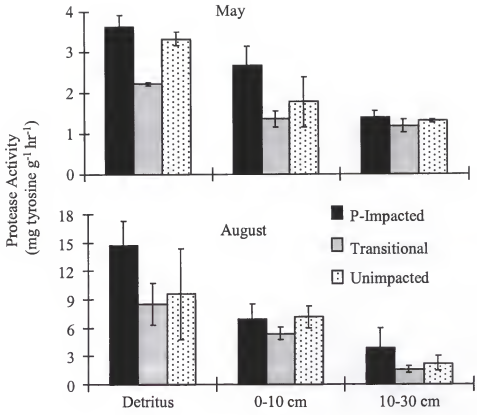

Figure 2.6. Protease activity (mg tyrosine g soil^{-1} hr^{-1}) in detritus and at two soil depths for the May and August 1996 sampling periods in P-impacted, transitional, and unimpacted areas. Error bars represent standard error.

bacterial populations (Mayer, 1989), and O_2 concentrations (McLatchey and Reddy, 1998). Protease activity varied seasonally, and was significantly higher ($P<0.05$) in August than in May. Protease activity was significantly negatively correlated with soil NH_4-N concentrations in the 0-10 cm soil depth ($r = -0.84$). At high substrate concentrations, some hydrolysis products, such as NH_4, are not assimilated by microorganisms and are released into soil solution; thus, an excess of NH_4 in solution at high substrate concentrations may repress some enzyme activities (Hoppe et. al., 1988).

Phenol Oxidase

Phenol oxidase activity was not affected by P loading in detritus and soil, with rates remaining constant along the P gradient in detritus and soil depths (Fig. 2.7). Phenol oxidase activity was highest in the detritus layer and decreased with depth at both sampling periods ($P<0.05$). Phenol oxidase often decreases with depth due to O_2 limitations (Pind et al., 1994; McLatchey and Reddy, 1998), but there were no seasonal variations in phenol oxidase activity. The presence of water on the soil surface may limit phenol oxidase activity (Benner et al., 1984). The phenol oxidase activity increased as particle size decreased, or as organic matter became more degraded (Jackson et al., 1995). Phenol oxidase activity in reclaimed wetlands was only detected under aerobic soil conditions at a rate of 1.3 mmole diqc kg^{-1} min^{-1} (McLatchey and Reddy, 1998). Phenol oxidase activity in northern peat soils ranged from 0.24 mmole diqc kg^{-1} min^{-1} at the soil surface to 0.05 mmole diqc kg^{-1} min^{-1} at 40 cm below surface (Pind et al., 1994).

Relationships between microbial parameters and extracellular enzyme activities are often contradictory because of a wide range of factors that influence enzyme

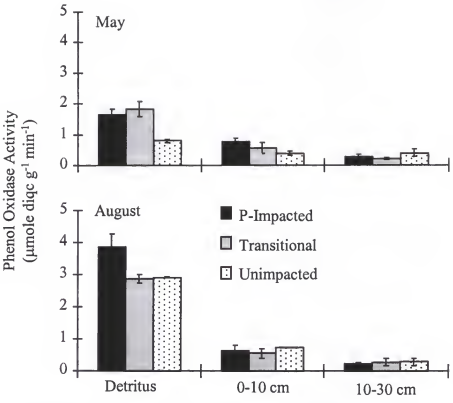

Figure 2.7. Phenol oxidase activity (µmole diqc g^{-1} min^{-1}) in detritus and at two soil depths for the May and August 1996 sampling periods in P-impacted, transitional, and unimpacted areas. Error bars represent standard error.

activities including redox conditions, nutrients, substrates, and other physico-chemical properties. Due to the complexity of enzyme and substrate interactions and the complimentary functions of many of these enzymes, relationships between enzyme activity and soil and microbial parameters are often not readily observed in the field (Marsden and Gray, 1986). In our study, sulfatase, glucosidase, and phenol oxidase activity were generally not significantly related to soil physico-chemical parameters. However, protease activity in soil appeared to be regulated, in part, by feedback inhibition and the presence of high NH_4 concentrations. Phosphatase activity, however, was sensitive to changes in soil and microbial P factors, suggesting that these factors were in part controlling APA activity.

Rates of extracellular enzyme activity in WCA-2a were often much greater than values reported in the literature for other systems. This difference suggests that detritus and soil in WCA-2a are both productive and biologically active; hence, enzyme activity may respond differently in this system than in others.

Conclusions

Phosphorus loading to an oligotrophic, P-limited wetland had a significant influence on APA in detritus and soil but not on other extracellular enzyme activities. Extracellular enzyme activity markedly decreased with depth in the soil profile, and detritus was most responsive to changes in P concentrations. The APA appeared to be regulated by specific soil and microbial P parameters in detritus and the upper soil depths. However, relationships between soil and microbial physico-chemical properties and other measured extracellular enzyme activities seldom produced significant

relationships. Alkaline phosphatase activity appeared to be suitable for use as an indicator of P eutrophication.

CHAPTER 3
HETEROTROPHIC MICROBIAL ACTIVITY IN NORTHERN EVERGLADES WETLAND SOILS

Introduction

Organic matter degradation plays an important role in nutrient cycling of wetlands. Rates of organic matter degradation and carbon dioxide (CO_2) production provide an indication of the microbial activity of soils, with primary factors influencing microbial activity including concentrations of utilizable substrates, electron acceptors, and nutrients, such as N and P (Webster and Benfield, 1986; D'Angelo and Reddy, 1994; Reddy et al., 1999).

A major limiting factor for microbial growth in short-term studies is the utilization of readily degradable compounds from the dissolved organic carbon (DOC) pool (Hoppe, 1983). Short-term studies primarily determine microbial respiration based on this pool. In long-term studies, utilizable portions of the DOC pool are depleted, and heterotrophic microbial activity measurements are often based on utilization of large organic compounds which first must be acted upon by extracellular enzymes, resulting in lower microbial activity (Chrost, 1991).

Organic matter degradation in wetlands is often limited by the availability of electron acceptors rather than electron donors (Reddy and D'Angelo, 1994; Amador and Jones, 1995; McLatchey and Reddy, 1998). Oxygen (O_2) is the most important electron acceptor in terrestrial systems, but is usually limited to the upper soil surface and the

overlying water column in wetlands. Sequential reduction of electron acceptors with depth in soils generally proceeds in the order O_2 > nitrate (NO_3) > sulfate (SO_4) > bicarbonates based on theoretical thermodynamic energy yields to microorganisms (Billen, 1982; Reddy and D'Angelo, 1994). Thus, degradation rates of DOC in wetland soils are higher under drained conditions with O_2 as the primary electron acceptor, and generally decrease in anaerobic environments depending on the availability of alternate electron acceptors (Lovley and Klug, 1986; McLatchey and Reddy, 1998; D'Angelo and Reddy, 1999).

The objectives of this study were to determine: 1) rates of potential heterotrophic microbial activities (represented by CO_2 and CH_4 production rates) under drained (aerobic) and flooded (anaerobic) conditions; and 2) the influence of added electron donors and electron acceptors on heterotrophic microbial activities.

Materials and Methods

Site Description

The study site was Water Conservation Area-2a (WCA-2a) of the Florida Everglades. The WCA-2a is a 447 km^2 wetland that has received nutrient-laden drainage waters for several decades from the adjacent Everglades Agricultural Area (EAA). Phosphorus loading during the past several decades has resulted in distinct gradients in P concentrations for the water column and soil (Reddy et al., 1993; DeBusk et al., 1994) from sites of inflow to the interior of the wetlands. Historically, this system was P limited and contained a mixture of sawgrass (*Cladium* sp.) and open slough communities (Davis, 1991). The addition of P-enriched inflow water and altered hydrology due to impoundment have been implicated in causing a vegetation shift from the indigenous

Cladium/slough communities to cattail (*Typha* sp.) dominated areas, resulting in increased soil accumulation rates and various soil and water alterations (Davis, 1991; Craft and Richardson, 1993; Reddy et al., 1993). Soils near the primary surface water inflow point S10-C exhibit the highest P concentrations, while soil P concentrations decrease with increasing distance from the inflow. There is also a shift in vegetative community type corresponding to changes in soil P concentrations along the P gradient (Miao and DeBusk, 1999). *Typha* is the dominant vegetation in areas impacted by P loading, while *Cladium*/sloughs dominate in P-unimpacted areas in the interior of WCA-2a (Davis, 1991; DeBusk et al., 1994).

Detritus and Soil Sampling and Characterization

Detritus and soil samples were collected from eight stations along a P gradient south from the primary point of inflow water (S10-C) of WCA-2a (Table 3.1). Sampling stations encompassed low and high concentrations of soil P (approximate range of 400-2000 mg P kg^{-1} soil) and three vegetative zones (*Typha*, mixed transitional area, and *Cladium*/sloughs). Detritus and soils were collected during August 1996 and March 1997 to represent the seasonal wet and dry seasons. Samples consisted of recently-deposited, readily distinguishable plant detritus on the soil surface and two soil depth intervals (0-10 and 10-30 cm). Detritus was collected at each of the sampling stations from above the cored soil. Soil cores were obtained by driving an aluminum corer (i.d.=14.6 cm) to a depth of approximately 40 cm. At each sampling station, four soil cores were obtained 1-2 m apart, with each soil core then being sectioned into 0-10 and 10-30 cm layers. Respective layers of all four cores were combined into one bulk sample and homogenized for use in experiments. A portion of samples was immediately used in field incubations

Table 3.1. Station locations along the nutrient gradient in Water Conservation Area-2a.

Station	Distance from Inflow (km)	Latitude N. (deg)	(min)	Longitude W. (deg)	(min)
P-Impacted					
1	1.40	26	21.53	80	21.20
2	2.27	26	21.05	80	21.21
3	3.25	26	20.53	80	21.27
Transitional					
4	4.19	26	20.02	80	21.37
5	5.12	26	19.52	80	21.39
6	6.98	26	18.51	80	21.46
Unimpacted					
7	8.41	26	17.73	80	21.42
8	10.13	26	16.81	80	21.48

as described below, while remaining samples were placed in plastic bags and stored on ice for use in laboratory experiments. Subsequently, all samples were stored at 4°C until analysis.

Soil properties were characterized from homogenized soil samples. Soil bulk density was determined on a dry weight basis (70°C). Soil total P was determined by nitric-perchloric acid digestion and analyzed using an ascorbic acid colorimetric procedure (Kuo, 1996). Total C and N concentrations in detritus and soil were determined on oven-dried (70°C), ground samples using a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Microbial biomass C was determined using a chloroform fumigation incubation/K_2SO_4 extraction procedure (Vance et al., 1987).

Influence of Drained and Flooded Conditions on Heterotrophic Microbial Activity

These experiments were designed to provide an estimate of *in situ* heterotrophic microbial activity (CO_2 production rates). Hence, experiments were initiated within 2 hr of sample collection and samples were incubated on-site in floodwater. To provide an estimate of field CO_2 production rates, a short-term incubation was utilized. Based on preliminary studies (data not presented), CO_2 production rates were linear over an 8 hr incubation period, so a 4 hr incubation period was selected.

Treatments included measurement of CO_2 production under drained and flooded soil conditions. For drained conditions, detritus and soil were placed onto glass fiber filters and excess water was drained for approximately 5 min. For flooded conditions, incubations were carried out using field-wet soil samples. Incubations were performed in triplicate and with controls to account for background CO_2 concentrations.

Wet season (August 1996)

This field study involved the measurement of CO_2 production under drained and flooded soil conditions. Schott media bottles with screw-type lids containing 10 g drained, moist soil and a NaOH trap were sealed under an atmosphere of 21% O_2 to facilitate aerobic conditions. A flooded (anaerobic) treatment containing 10 g wet samples of flooded soil with a N_2 headspace was also included. For substrate-induced respiration (SIR) measurements, both drained and flooded treatments were supplemented with glucose at an excess concentration of 25 mg C g^{-1}, based on results of previous experiments (DeBusk, 1996). Incubations were carried out at ambient floodwater temperature (28 ± 2°C).

Carbon dioxide production was quantified using an alkali trap method in which 10 mL of 0.1 *M* NaOH was added to small vials placed inside incubation bottles. After 4 hr of incubation, NaOH traps were removed and capped. The CO_2 trapped in NaOH was subsequently analyzed by titration with acid (Zibilske, 1994). For titrations, $BaCl_2$ was added to the traps and remaining NaOH was titrated with 0.05 *M* HCl to the phenolphthalein endpoint. After incubation, detritus and soil samples were oven-dried at 70°C to determine sample dry weight per incubation bottle.

Dry season (March 1997)

The experimental design involved measurements taken within 2 hr of sample collection (basal CO_2 production) and a later incubation with added substrates (SIR) initiated approximately 6 hr after sample collection. Incubations for basal CO_2 production were carried out at ambient floodwater temperature (28°C) for a period of 4 hr. The experimental design was similar to that described for the wet-season experiment.

Following measurement of basal CO_2 production, additional incubations were initiated on the same samples to determine SIR. Substrate sources included either a mixture of C sources (glucose, citrate, malate, oxalate, and acetate; each component contributing 20% of the total C in the mixture) or a C, N, S, and P source (peptone) at rates of 25 mg C g $soil^{-1}$. Both substrate solutions were buffered to pH=7 and autoclaved. After completion of field incubations, substrate solutions was added to incubation bottles, a NaOH trap (3 mL of 0.2 *M*) was added, and samples were either incubated under an atmosphere of 21% O_2 or were purged with N_2. After a 4 hr incubation at 28°C, NaOH traps were removed and sealed with caps fitted with rubber septa.

Unlike the previous study during the wet season, CO_2 trapped in NaOH was determined by gas chromatography after acidification of the NaOH. This method was found to be more sensitive to levels of CO_2 than the titration method. To the enclosed NaOH in vials, excess HCl was added through septa to neutralize the NaOH and result in a pH<2.0. A portion of the resulting CO_2 in the headspace was sampled by syringe for injection to a gas chromatograph. Carbon dioxide was measured using a Shimadzu GC-8A gas chromatograph fitted with a thermal conductivity detector (30°C), He as carrier gas, and a 0.3 cm by 2 m Poropak N column (Supelco Inc., Bellefonte, PA) at 25°C. Gas pressure in the vials was measured using a digital pressure meter (Kane-May, UK). Carbon dioxide trapped in the NaOH was then calculated using a modification of a method described by (Martens, 1987) in which the universal gas law, pressure in the vials, solution pH, CO_2 concentration in the headspace, and solution and headspace volumes were used to calculate trapped CO_2.

Influence of Added Inorganic Electron Acceptors on Heterotrophic Microbial Activity

This experiment utilized detritus, 0-10 cm, and 10-30 cm soil sampled from 8 stations along the P gradient in WCA-2a during March 1997 (dry season). Four treatments (6 replicates per treatment) were applied to each soil depth at each station. Treatments included incubation under aerobic, NO_3-reducing, SO_4-reducing, and methanogenic conditions. The electron acceptors, NO_3 and SO_4, were added on an electron equivalent basis and at concentrations determined in preliminary experiments to not become limiting during the incubation period. A KNO_3 solution was added to soil resulting in an initial concentration of 1600 μg NO_3-N g^{-1} dry soil. A K_2SO_4 solution was added to soil resulting in initial concentration of 2300 μg SO_4-S g^{-1} dry soil.

Approximately 10 g wet soil was added to incubation bottles followed by addition of electron acceptors. For O_2 treatments, samples were drained on glass fiber filters to remove excess water and to allow for aerobic conditions. A vial containing 5 mL of 0.1 *M* NaOH was placed inside each incubation bottle to trap evolved CO_2. Bottles were purged with N_2 gas for all treatments with the exception of the O_2 treatment. For the O_2, NO_3, and SO_4-reducing treatments, the NaOH traps were removed from incubation bottles at 4, 8, 16, and 24 hr and then at 2 d intervals up to 10 d. For the methanogenic CO_2-production treatments, NaOH traps were sampled at 4, 8, 16, and 24 hr and then periodically up to 40 d. At the end of each sampling period, bottles from anaerobic treatments were purged with N_2. The NaOH traps then were analyzed for CO_2 by gas chromatography after acidification. Methane (CH_4) accumulation in the headspace was monitored by periodic sample headspace injection into a Shimadzu gas chromatograph-8A fitted with flame ionization detector (160°C), N_2 as the carrier gas, and a 0.3 cm by

2 m Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 160°C. Controls were included to account for background CO_2 and CH_4.

Data Analysis

Comparisons of treatments and significant differences were determined using one-way and three-way Analysis of Variance (ANOVAs) with a Fisher's LSD at $P<0.05$ (CoStat, Minneapolis, MN). A completely randomized experimental design was utilized with factors including electron-acceptor additions, sampling stations, soil depth, or season. Correlation coefficients were determined using CoStat. Field replications were developed by combining adjacent sampling stations into distinct groups based on soil P concentrations and vegetation type. The groupings for site comparisons were: stations 1-3 (P-impacted area growing *Typha*) stations 4-6 (transitional area containing both *Typha* and *Cladium*), and stations 7-8 (P-unimpacted area growing *Cladium*). Statistical differences at $P<0.05$ were compared between the P-impacted, transitional, and unimpacted areas.

Results

Characterization of Detritus and Soil

The pH of detritus and soil did not significantly change along the P gradient and was in the range 7.0 to 7.8 (data not presented). Soil bulk density increased with increasing depth but did not vary along the P gradient (Table 3.2). Detritus and soil total P concentrations were highest in the impacted area and decreased toward the transitional and unimpacted area. Total P also decreased as a function of soil depth. Total C and N contents of detritus and soil were not affected by P loading and generally did not vary with soil depth. Microbial biomass C was highest in the surface detritus layer and

Table 3.2. Soil physico-chemical parameters in detritus and soil from the nutrient gradient in Water Conservation Area-2a for two sampling periods. Values in parenthesis are one standard deviation. The P-impacted area included stations 1-3, the transitional area included stations 4-6, and the unimpacted area included stations 7-8.

Depth	Sites	Bulk Density	Total P	Total C	Total N	Microbial Biomass C
Wet Season		$g\ cm^{-3}$	$mg\ P\ kg^{-1}$	----------	$g\ kg^{-1}$	----------
Detritus	P-Impacted	nd	1390 (63)	461 (20)	22 (4)	19 (6)
	Transitional	nd	964 (103)	450 (3)	21 (4)	9 (4)
	Unimpacted	nd	513 (148)	419 (11)	24 (6)	6 (3)
0-10 cm	P-Impacted	0.05 (0)	1270 (158)	436 (19)	26 (1)	2 (1)
	Transitional	0.07 (0)	769 (261)	429 (8)	27 (1)	1 (1)
	Unimpacted	0.07 (0)	405 (13)	434 (4)	28 (1)	2 (1)
10-30 cm	P-Impacted	0.20 (0)	380 (99)	454 (14)	30 (2)	1 (1)
	Transitional	0.19 (0)	227 (48)	432 (33)	26 (4)	1 (0)
	Unimpacted	0.17 (0)	211 (1)	469 (4)	26 (0)	1 (1)
Dry Season						
Detritus	P-Impacted	nd	1780 (136)	427 (6)	27 (1)	14 (2)
	Transitional	nd	1490 (431)	397 (33)	23 (2)	17 (3)
	Unimpacted	nd	569 (175)	402 (33)	26 (0)	10 (11)
0-10 cm	P-Impacted	0.07 (0)	1430 (132)	411 (29)	28 (2)	6 (3)
	Transitional	0.08 (0)	869 (110)	412 (16)	28 (4)	6 (4)
	Unimpacted	0.06 (0)	619 (4)	421 (3)	27 (2)	8 (3)
10-30 cm	P-Impacted	0.11 (0)	445 (93)	458 (13)	31 (3)	2 (1)
	Transitional	0.09 (0)	276 (43)	383 (105)	25 (5)	2 91)
	Unimpacted	0.08 (0)	255 (25)	458 (2)	30 (4)	1 (0)

nd = not determined

decreased with soil depth and was also higher at P-impacted area than at the unimpacted area.

Field Experiments

Influence of drained and flooded conditions on heterotrophic microbial activity: wet season

Substrate-induced respiration rates were highest in P-impacted areas and decreased along the P gradient to the unimpacted areas ($P<0.05$). This occurred for all three soil depth intervals and also under both drained and flooded conditions (Table 3.3). Basal CO_2 production rates under drained conditions were highest for detritus and significantly ($P<0.05$) decreased with soil depth (Fig. 3.1). Soil CO_2 production rates for 0-10 cm soil under drained conditions were significantly greater than rates under flooded conditions. However, an opposite trend was observed for the 10-30 cm soil depth. The CO_2 production rates in flooded 10-30 cm soil were generally greater than rates under drained conditions, although differences were not significant.

Substrate-induced (glucose added) CO_2 production rates under drained conditions were highest in the detritus layer and significantly ($P<0.05$) decreased with depth (Table 3.3). Addition of glucose significantly increased ($P<0.05$) CO_2 production under drained conditions in the detritus layer but had no effect in the 0-10 and 10-30 cm layers or under flooded conditions. In the 0-10 cm layer, CO_2 production rates under drained conditions were significantly greater than rates under flooded conditions either with or without glucose addition.

Table 3.3. Substrate-induced CO_2-production rates (standard error) in detritus and soil along the nutrient gradient in Water Conservation Area-2a. The P-impacted area included stations 1-3, the transitional area included stations 4-6, and the unimpacted area included stations 7-8.

Season	Soil Depth	Treatment	Substrate	P-Impacted	Transitional	Unimpacted
	(cm)			---------- mg CO_2-C kg^{-1} hr^{-1} ----------		
Wet	Detritus	Drained	Glucose	188 (7)	143 (1)	117 (19)
	0-10	Drained	Glucose	162 (12)	104 (24)	83 (11)
		Flooded	Glucose	107 (20)	78 (24)	59 (13)
	10-30	Drained	Glucose	88 (35)	61 (7)	48 (10)
		Flooded	Glucose	87 (5)	99 (42)	56 (20)
Dry	Detritus	Drained	C sources	57 (2)	67 (6)	59 (13)
		Drained	Peptone	128 (13)	114 (22)	137 (5)
		Flooded	C sources	62 (13)	42 (3)	45 (5)
		Flooded	Peptone	106 (10)	70 (13)	60 (6)
	0-10	Drained	C sources	54 (11)	29 (10)	50 (14)
		Drained	Peptone	52 (5)	23 (4)	57 (11)
		Flooded	C sources	50 (4)	18 (4)	25 (1)
		Flooded	Peptone	47 (4)	22 (1)	23 (2)
	10-30	Drained	C sources	21 (6)	15 (2)	30 (11)
		Drained	Peptone	27 (1)	27 (10)	28 (8)
		Flooded	C sources	26 (7)	18 (3)	11 (0)
		Flooded	Peptone	14 (6)	28 (6)	14 (1)

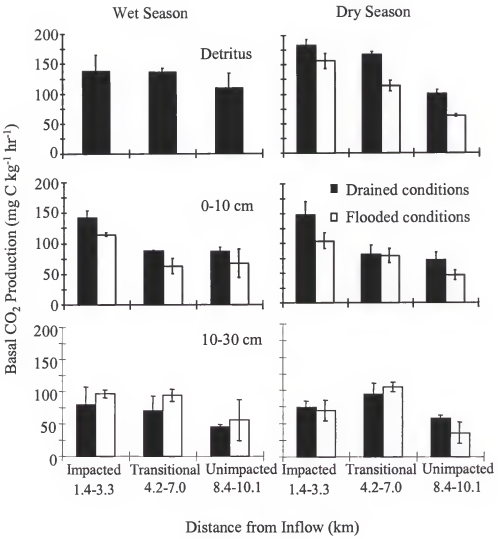

Figure 3.1. Basal CO_2 production rates in drained and flooded detritus and soil along the nutrient gradient in Water Conservation Area-2a for two sampling periods.

Influence of drained and flooded conditions on heterotrophic microbial activity: dry season

Basal CO_2 production rates were highest in detritus and significantly ($P<0.05$) decreased with depth under both drained and flooded conditions (Fig. 3.1). Production rates in drained samples were greater ($P<0.05$) than rates in flooded samples for both detritus and 0-10 cm soil. However, no differences in CO_2 production rates between drained and flooded conditions were observed in the 10-30 cm depth. Both drained and flooded basal CO_2 production rates significantly ($P<0.05$) decreased along the P gradient in the detritus and in the 0-10 cm depth but not in the 10-30 cm depth.

Under drained conditions, addition of peptone increased CO_2 production rates more than the mixture of C sources in the detritus ($P<0.05$) (Table 3.3). Production rates of CO_2 under drained conditions were highest in the detritus and decreased with depth regardless of which substrates were added. Under flooded conditions, addition of peptone increased CO_2 production more than addition of C sources but only in the detritus ($P<0.05$). Flooded-soil CO_2 production rates were highest in the detritus and decreased with depth regardless of which substrate was added. Drained soil CO_2 production rates were greater than flooded rates in detritus and in 0-10 cm layer with either substrate added. No seasonal differences in CO_2 production rates were observed in detritus and soil.

Basal CO_2 production rates measured during the wet and dry season experiments were significantly ($P<0.05$) related to the total P content of detritus and soil (Fig. 3.2). Anaerobic CO_2 production rates were approximately 64% of the aerobic rates (Fig. 3.3).

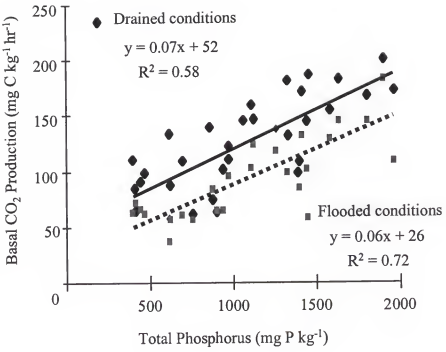

Figure 3.2. Basal CO_2 production rates in detritus and soil from two sampling periods plotted against total P. Data points are means from drained and flooded treatments.

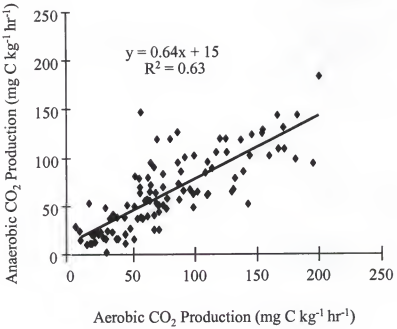

Figure 3.3. Basal and substrate-induced CO_2-production rates under drained conditions plotted against production rates under flooded conditions. Data points represent two sampling periods.

Influence of Added Inorganic Electron Acceptors on Heterotrophic Microbial Activity

Cumulative CO_2 production in soils amended with various inorganic electron acceptors showed two distinct phases: 1) an initial rapid phase involving decomposition of labile soluble organic compounds; and 2) a slower rate of decomposition of polymeric organic compounds. Both rates were described by a linear relationship, where k_1 was the phase I decomposition rate and k_2 was the phase II decomposition rate, both rates being in mg CO_2-C kg^{-1} hr^{-1}.

Heterotrophic microbial activity was generally highest at the P-impacted area and decreased at unimpacted areas (Figs. 3.4 and 3.5). Differences among various electron-acceptor treatments were also evident. Aerobic respiration was significantly ($P<0.05$) higher than all other modes of respiration and methanogenesis. Differences with soil depth were also observed, with CO_2 and CH_4 production rates being higher in detritus than in underlying soil.

For all processes except CH_4 production, the first phase of the microbial respiration rate (k_1) was significantly greater than the second phase (k_2). However, for CH_4 production, k_2 values were significantly greater than k_1 values in detritus and 0-10 cm soil, though $k_1 = k_2$ in 10-30 cm soil (Table 3.4). In detritus and both soil depths, no significant changes in aerobic or NO_3-reducing k_1 or k_2 values were evident along the P gradient (Table 3.4). However, under SO_4-reducing conditions, detritus and 0-10 cm soil in unimpacted areas had lower k_1 and k_2 values than in P-impacted areas.

When k_1 and k_2 data were combined over detritus and both soil depths, several additional relationships were observed. Under aerobic conditions, P-impacted areas had higher k_2 values than unimpacted areas. Under NO_3- and SO_4-reducing conditions,

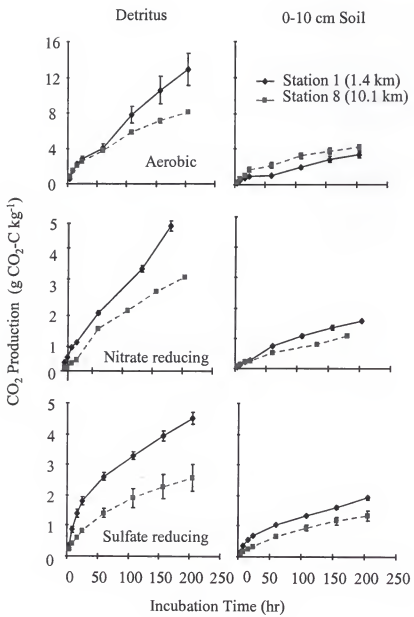

Figure 3.4. Heterotrophic microbial activity in detritus and 0-10 cm soil at 1.4 km (station 1) and 10.1 km (station 8) from the inflow in Water Conservation Area-2a. Data are presented for aerobic, nitrate-reducing, and sulfate-reducing conditions.

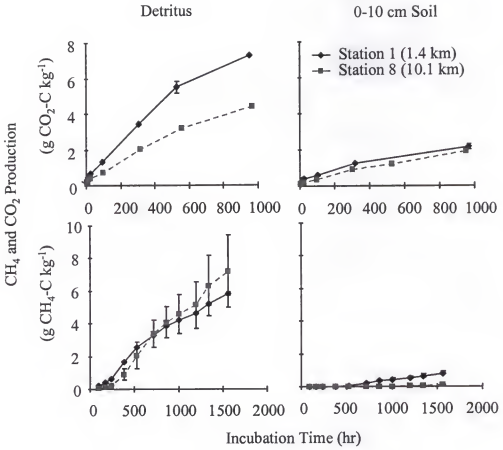

Figure 3.5. Heterotrophic microbial activity in detritus and 0-10 cm soil at 1.4 km (station 1) and 10.1 km (station 8) from the inflow in Water Conservation Area-2a. Data are presented for CO_2 and CH_4 production rates for methanogenic conditions.

Table 3.4. Carbon dioxide and CH_4-production rates (standard error) in detritus and soil in P-impacted, transitional, and unimpacted areas along the nutrient gradient in Water Conservation Area-2a. Data are presented for the k_1 and k_2 phases.

Soil Depth	Treatment	P-Impacted	Transitional	Unimpacted
		----------mg CO_2-C kg^{-1} hr^{-1}----------		
k_1 Phase				
Detritus	Aerobic	97.7 (11.6)	101.8 (21.5)	83.7 (18.7)
	Denitrifying	39.3 (1.0)	32.8 (7.5)	19.6 (5.1)
	Sulfate Reducing	61.7 (7.5)	46.4 (7.6)	33.0 (0.6)
	Methanogenic CO_2	59.0 (17.5)	51.1 (14.7)	33.6 (10.8)
	Methanogenic CH_4	1.7 (0.7)	1.8 (1.0)	1.5 (0.6)
0-10 cm	Aerobic	36.0 (3.7)	43.8 (17.2)	50.2 (16.9)
	Denitrifying	16.8 (4.2)	9.6 (4.0)	12.1 (0.1)
	Sulfate Reducing	25.1 (2.9)	13.5 (3.8)	13.7 (0.8)
	Methanogenic CO_2	23.4 (1.5)	26.2 (9.9)	28.9 (1.5)
	Methanogenic CH_4	0.2 (0.1)	0.1 (0.1)	0.0 (0.0)
10-30 cm	Aerobic	8.6 (1.4)	7.0 (0.7)	8.9 (2.2)
	Denitrifying	4.0 (1.4)	2.4 (0.7)	2.1 (0.5)
	Sulfate Reducing	5.9 (1.3)	4.1 (0.8)	2.9 (0.6)
	Methanogenic CO_2	7.7 (1.6)	5.8 (1.5)	4.9 (0.6)
	Methanogenic CH_4	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
k_2 Phase				
Detritus	Aerobic	46.1 (6.5)	40.0 (4.1)	26.9 (4.7)
	Denitrifying	19.7 (2.6)	18.2 (1.6)	15.4 (1.1)
	Sulfate Reducing	11.8 (1.8)	16.0 (1.1)	10.5 (0.9)
	Methanogenic CO_2	7.2 (0.3)	6.2 (0.2)	5.1 (0.7)
	Methanogenic CH_4	3.6 (0.3)	3.3 (0.6)	4.9 (0.3)
0-10 cm	Aerobic	15.2 (1.3)	11.0 (2.1)	12.4 (2.1)
	Denitrifying	7.3 (1.5)	5.8 (2.5)	7.0 (0.2)
	Sulfate Reducing	6.0 (0.5)	4.3 (1.3)	5.2 (0.4)
	Methanogenic CO_2	1.5 (0.3)	1.9 (0.7)	2.3 (0.4)
	Methanogenic CH_4	1.3 (0.4)	0.1 (0.1)	1.0 (0.9)
10-30 cm	Aerobic	2.8 (0.1)	2.3 (0.1)	2.6 (0.5)
	Denitrifying	1.8 (0.4)	1.5 (0.4)	1.3 (0.1)
	Sulfate Reducing	1.6 (0.2)	1.7 (0.5)	1.3 (0.4)
	Methanogenic CO_2	0.7 (0.0)	0.7 (0.1)	0.8 (0.1)
	Methanogenic CH_4	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)

impacted areas had higher k_1 values than unimpacted areas. The k_1 values were highest in impacted areas and significantly decreased in transitional areas before decreasing again in unimpacted areas. Contrary to other electron-acceptor treatments where k_1 and k_2 values tended to decrease along the P gradient, k_2 values for CH_4 production were highest at unimpacted areas.

Soil depth had a strong effect on both k_1 and k_2 values. Under aerobic, NO_3-reducing, SO_4-reducing, and methanogenic-CO_2 producing conditions, both k_1 and k_2 values were significantly ($P<0.05$) highest in detritus and decreased with depth (Table 3.4). However, CH_4 production exhibited slightly different relationships with depth. Although CH_4-production k_1 and k_2 values were significantly higher in detritus, there were no differences in k_1 and k_2 values between 0-10 cm and 10-30 cm soil.

The k_1 and k_2 values under aerobic conditions was significantly ($P<0.05$) greater than k_1 and k_2 values for all other electron-acceptor treatments in detritus, 0-10 cm, and 10-30 cm soil (Table 3.4). The k_1 values for CH_4 production were significantly ($P<0.05$) lower than values for all other treatments in both detritus and soil. The k_1 values under denitrifying and SO_4-reducing conditions were similar in detritus but k_1 values were significantly ($P<0.05$) higher under SO_4-reducing conditions than under denitrifying conditions in 0-10 cm soil.

For k_2 values, no differences between NO_3- and SO_4-reducing conditions were observed in any soil depth. However, k_2 values under NO_3- and SO_4-reducing conditions were significantly greater than methanogenic CO_2 and CH_4-producing conditions for all soil depths. The k_1 and k_2 values for aerobic conditions were plotted against values for other electron-acceptor treatments (Figs. 3.6 and 3.7), with CO_2 production under NO_3-

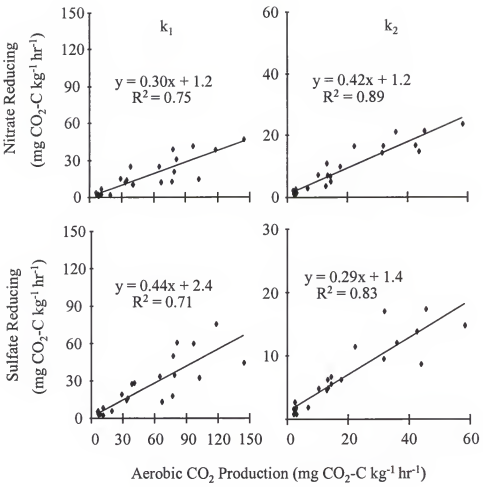

Figure 3.6. Aerobic CO_2-production rates plotted against production rates under nitrate and sulfate-reducing conditions for the k_1 and k_2 phases.

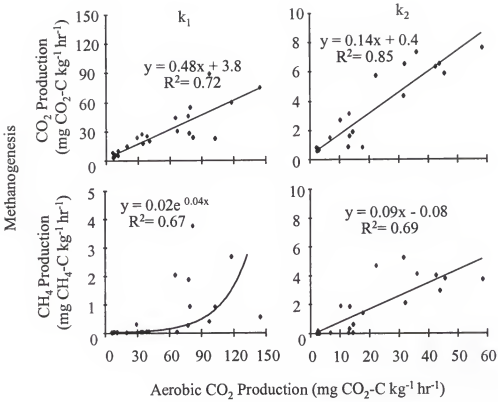

Figure 3.7. Aerobic CO_2-production rates plotted against CO_2 and CH_4-production rates for methanogenic conditions for the k_1 and k_2 phases.

reducing conditions being 30% of aerobic production for the k_1 phase, but increasing to 42% for the k_2 phase. The SO_4 reduction rate was 44% of the aerobic rate for the k_1 phase but decreased to 29% for the k_2 phase. The CH_4-production rates were only 9% or less of the aerobic CO_2-production rates.

Discussion

Heterotrophic microbial activities were generally highest in P-impacted areas and decreased in unimpacted areas. This decreasing trend occurred primarily in detritus and in the 0-10 cm soil layer, corresponding to decreases in soil P concentrations. Several studies have shown that organic matter degradation is greater at higher P levels (Amador and Jones, 1993; Westermann, 1993; Amador and Jones, 1995; DeBusk, 1996). In our study, positive correlations were obtained between heterotrophic microbial activities and soil total P.

Basal and substrate-induced CO_2-production rates decreased with depth in the soil profile, also corresponding to decreases in extracellular enzyme activity and in substrate quality with depth (DeBusk and Reddy, 1998; Wright and Reddy, 2001a). Many factors, including dissolved O_2, enzyme activity, electron acceptor concentrations, and substrate quality, also decreased with soil depth (McKinley and Vestal, 1992; D'Angelo and Reddy, 1994; DeBusk, 1996). Substrate quality can be expressed using a lignocellulose index (LCI), which is the ratio of lignin to lignocellulose (Melillo et al., 1989). Lignin content in Everglades soils increased with depth in conjunction with decreases in cellulose content; thus, substrate quality decreased with depth and has been implicated as an important factor regulating organic matter degradation (Swift et al., 1979; Benner et al., 1984; DeBusk, and Reddy, 1998). Soil depth had a strong effect on both k_1 and k_2

values in our laboratory studies. Under aerobic, denitrifying, SO_4-reducing, and methanogenic-CO_2 producing conditions, both k_1 and k_2 values were significantly ($P<0.05$) highest in detritus and decreased with depth (Table 3.4). Thus, substrate quality may be a primary controlling factor of heterotrophic microbial activities in subsurface wetland soils.

Oxygen appeared to regulate organic matter degradation in these studies, with CO_2-production rates for drained soil being higher than for flooded soil. This was expected as microbial activity is normally greater under aerobic conditions (Benner et al., 1984; Bridgham and Richardson, 1992; DeBusk and Reddy, 1998; McLatchey and Reddy, 1998, D'Angelo and Reddy, 1999). In wetland soils, degradation generally proceeds via a thermodynamically-favored sequential reduction of the electron acceptors O_2, NO_3, SO_4, and HCO_3 (Zehnder and Stumm, 1988; Reddy and D'Angelo, 1994). Based on the theory of preferential use of electron acceptors by microorganisms, aerobic microbial activities should be greater than anaerobic activities since more energy can be obtained by microorganisms when using O_2. In the short-term incubation field experiments, the anaerobic CO_2 production rate was 64% of the aerobic rate (Fig. 3.3), which was somewhat higher than similar rates measured under laboratory conditions (Bridgham and Richardson, 1992; DeBusk and Reddy, 1998). In our short-term incubation of 4 hr, O_2 contamination during soil mixing may have contributed to an inflated anaerobic CO_2-production rate. DeBusk and Reddy (1998) reported that anaerobic CO_2-production rates were 32% of the aerobic rates along the same P gradient in WCA-2a. Similar results have been reported to range from 34-63% (Bridgham and Richardson, 1992) and 37% (Benner et al., 1984). Soils along the P gradient often

exhibit low NO_3 concentrations; thus, upon initiation of experiment, population sizes of denitrifiers were likely low. After an adjustment period during the k_1 phase, NO_3-reducers likely increased in population size, thus increasing CO_2 production up to 42% of aerobic CO_2-production rates during the k_2 phase.

In these experiments, the sequential reduction of electron acceptors held true for O_2 (highest rates) and HCO_3^- (lowest rates), but not for NO_3 and SO_4. In most cases, there were no differences in heterotrophic microbial activities under NO_3- and SO_4-reducing conditions, perhaps due to the presence of SO_4 and a general lack of NO_3 in soils along most of the P gradient. It was expected that CO_2 production rates would be greater under NO_3- rather than SO_4-reducing conditions. Perhaps relatively higher SO_4 concentrations and lower NO_3 concentrations in soils along the P gradient tended to support SO_4 reduction and limit denitrification. The decrease in SO_4-reduction rates along the P gradient during both the rapid k_1 phase and the more stable k_2 phase may be due to P loading or the presence of high SO_4 concentrations near the inflow point. Sulfate concentrations have been detected at high levels along the P gradient (Schipper and Reddy, 1995), and population sizes of SO_4 reducing bacteria were enhanced by P (Drake et al., 1996).

Addition of C substrates in field experiments tended to increase CO_2 production rates but only in the detritus. Addition of peptone increased CO_2 production in several cases compared to addition of a C source only. Peptone is a mixture of various compounds containing C, N, P, S, and micronutrients, so it appears that addition of nutrients other than C stimulated CO_2 production. Since peptone contains both N and P, either or both of these nutrients may be responsible for increased CO_2 production

compared to treatments receiving only C sources. However, since P concentrations were already high at impacted areas, and since peptone increased CO_2 production rates in these areas, it seems unlikely that additional P in the form of peptone would be responsible for increased CO_2-production rates. Amador and Jones (1995) showed that additions of C and P increased C mineralization rates in P limited areas of the Everglades but not in areas of P enrichment. The N supplied by peptone may be responsible for the increased CO_2-production rates observed in P-impacted areas. Indeed, N may become limiting to heterotrophic microbial activity in areas of high P concentrations (DeBusk, 1996).

Heterotrophic microbial activity measured in field and laboratory experiments utilizing various electron acceptors was significantly ($P<0.05$) correlated with many soil physical and chemical parameters, including soil total P, extractable TOC, and microbial biomass C. Heterotrophic microbial activity has been reported in several studies to be enhanced by P additions or in high P soils (Bridgham and Richardson, 1992; Amador and Jones, 1993; Amador and Jones, 1995; DeBusk and Reddy, 1998). Extractable TOC represents the most utilizable portion of soil total C, and thus was expected to be related to CO_2 and CH_4-production rates. However, soil total C was not related to heterotrophic microbial activity, suggesting that the bulk of soil total C was not utilizable. Carbon dioxide production rates in drained detritus and soil were significantly ($P<0.05$) correlated with glucosidase activity (Wright and Reddy, 2001a). The significant relationships between enzyme activity and heterotrophic microbial activity in drained soil suggests that enzyme activity was closely coupled with microbial CO_2 and CH_4 production in Everglades soil, most likely during the longer-term k_2 phase. However, anaerobic CO_2 production rates and enzyme activities were not significantly correlated.

Heterotrophic microbial activity was significantly enhanced by the draining of soil and its exposure to O_2. This has important implications with regard to water management, organic matter degradation, and nutrient cycling. Exposure of soil to O_2, such as occurs during periods of low rainfall or low water inputs, increases heterotrophic microbial activity. This increase in microbial activity contributed to enhanced organic matter degradation rates and increased regeneration and cycling of nutrients in wetlands, which could potentially lead to increased nutrient concentrations in the water column.

Conclusions

Heterotrophic microbial activity was higher under drained conditions than under flooded conditions. Activity was also highest under aerobic conditions that decreased when other electron acceptors were utilized, suggesting that during periods of low water or dry down in WCA-2a, organic matter degradation and nutrient regeneration would be enhanced. Heterotrophic microbial activity may be C limited, in addition to being limited by nutrients such as N or P. Heterotrophic microbial activities in both detritus and surface soil were enhanced in P-impacted areas and were positively correlated with soil P parameters. Thus, these activities may serve well as indicators of eutrophication or of shifts in microbial dynamics due to P loading. The detritus was most responsive to P loading and thus showed greater potential for early response to P loading. Heterotrophic microbial activity and its controlling factors, such as electron-acceptor levels, play an important role in the regulation of organic matter degradation and subsequent nutrient regeneration.

CHAPTER 4
MICROBIAL UTILIZATION OF ELECTRON DONORS IN EVERGLADES SOIL

Introduction

Organic matter degradation in soil is dependent on microbial biomass, concentration of available nutrients, organic substrates, and various environments factors including temperature, moisture content, and redox potential (Webster and Benfield, 1986; Benner et al., 1994; Amador and Jones, 1995; D'Angelo and Reddy, 1999). Heterotrophic microbial activity is often enhanced by additions of limiting nutrients or C substrates (Chrost, 1991; McKinley and Vestal, 1992; Amador and Jones, 1993: Amador and Jones, 1995; Drake et al., 1996). In wetland soils, availability of electron acceptors is considered the primary limiting factor of organic matter degradation and heterotrophic microbial activity (D'Angelo and Reddy, 1999).

Readily utilizable or labile C may be limiting heterotrophic microbial activity in wetland soils (Bachoon and Jones, 1992; D'Angelo an d Reddy, 1999; Wright and Reddy, 2001b). Most of the C in wetland soils is present as ligno-cellulose, as lignin, or as other C fractions exhibiting varying degrees of recalcitrance (DeBusk, 1996). Most of the DOC pool contains large-molecular-weight compounds, and 90% of dissolved organic matter is considered refractory (Degens and Mopper, 1976; Chrost, 1991). Once the readily utilizable portions of the DOC pool are depleted, extracellular enzymes must convert the large-molecular-weight compounds into labile organic C.

Organic matter degradation in soils is a complex process involving both aerobic and anaerobic microorganisms. During short-term incubations, organic matter degradation degradation is based on microbial utilization of labile portions of the DOC pool. Long-term incubations are based on the conversion of more recalcitrant C substrates into labile organic C. Hence, carbon dioxide (CO_2) and methane (CH_4) production rates are often much higher during the first few days of incubation, with rates of CO_2 and CH_4 production then decreasing over time as the labile organic C pool is depleted (Wright and Reddy, 2001b). Polysaccharides and amino acids derived from the breakdown of lignin, ligno-cellulose and detrital tissue are utilized in microbial respiratory pathways (Chrost, 1991). Various carboxylic acids and fatty acids serve as substrates for anaerobic microorganisms in wetland soils (Lovley and Klug, 1982; Stams, 1994). Further degradation of these substrates by both aerobic and fermentative microorganisms leads to the production of alcohols, carboxylic acids, and inorganic nutrients. Eventually, these compounds are broken down to either CO_2 or CH_4 or are retained in microbial biomass.

Functional microbial diversity in soil has been characterized by measuring the short-term response of microbial communities to addition of a wide variety of C substrates (Degens and Harris, 1997; Hitzl et al., 1997; Degens, 1999). Substrate induced respiration is often dependent on the size of the microbial biomass pool (Sparling and Williams, 1986; Sparling and West, 1998). However, response of the microbial communities to added electron donors may not be entirely dependent on microbial biomass, but may be related as well to the catabolic diversity of soil microorganisms (Degens and Harris, 1997).

Additions of inorganic N and P and of various electron donors have been shown to enhance CO_2 and CH_4 production in Everglades soil (Amador and Jones, 1995; Wright and Reddy, 2001b). In addition, rapid utilization of added electron donors provides an indication of previous microbial exposure to particular electron donors as well as the presence of extracellular enzymes and other degradation pathways (Degens and Harris, 1997, Degens, 1998). The longer-term utilization of added electron donors provides an indication of the enzymatic capability of soil microorganisms to utilize electron donors and thus may be more dependent on soil microbial biomass. The objective of this study was to determine the response of anaerobic soil heterotrophic microbial communities to additions of various electron donors and limiting nutrients.

Materials and Methods

Site Description

The study area is located in Water Conservation Area-2a (WCA-2a) of the Florida Everglades. The WCA-2a is a 447 km^2 impounded wetland that has received nutrient-laden drainage waters for several decades from the adjacent Everglades Agricultural Area (EAA). Inflow of these waters has resulted in increased phosphorus (P) concentrations in the soil and in the overlying water column and the development of a distinct gradient in soil total P south of the primary water inflow point S10-C (Reddy et al., 1993; DeBusk et al., 1994; DeBusk et al., 2001a) into the interior of WCA-2a. Historically, this wetland was P-limited and contained a mixture of sawgrass (*Cladium* sp.) and slough communities (Davis, 1991). The addition of P-enriched waters and of altered hydrology due to impoundment have been implicated in causing a vegetation shift from the indigenous *Cladium*/slough communities to cattail (*Typha* sp.) dominated areas, resulting

in increased peat accumulation and additional soil and water alterations (Davis, 1991; Craft and Richardson, 1993; Reddy et al., 1993). Soils near the water inflow exhibit the highest P concentrations, which then decrease with increasing distance from the inflow. Corresponding to changes in soil P concentrations along the gradient, there is a shift in vegetative community type from areas near water inflow points to the interior of the wetland (Miao and DeBusk, 1999). *Typha* is the dominant vegetative type in areas impacted by P loading, while *Cladium*/sloughs are common in unimpacted areas (Davis, 1991; DeBusk et al., 2001a).

Detritus and Soil Sampling and Characterization

Experiments were conducted on detritus and soil samples collected during March 1999 and June 2001 in WCA-2a. During 1999, detritus and soil samples were collected from 3 stations (W2, W5, and W8) along a P gradient 2.3, 5.1, and 10.1 km south from the primary point of water inflow (S10-C) to WCA-2a. Sampling stations encompassed a range of TP concentrations (approximately 400-2000 mg P kg^{-1} soil) and different vegetative zones (*Typha*, mixed area, *Cladium*/sloughs). Samples consisted of recently-deposited, readily-distinguishable surface plant detritus and a soil depth interval from 0-10 cm. Detritus was collected at each of the sampling stations from above the cored soil. Soil cores were obtained by driving an aluminum corer (i.d.=14.6 cm) to a depth of approximately 40 cm. Triplicate soil cores were obtained at each site and were stored at 4°C until analysis. Cores were then sectioned to obtain the 0-10 cm surface layer.

In June 2001, only 0-10 cm soil samples were collected from a P-impacted site (1.8 km) and an P-unimpacted interior reference site (10.8 km) south of the water-inflow point S10-C of WCA-2a. The 0-10 cm soil was collected as described previously.

However, soil from the reference interior site contained large quantities of living detrital calcareous periphyton that were present on the soil surface due to low water levels.

Short-Term Response of Ambient Soil Microbial Community

These batch experiments were designed to provide an estimate of the short-term response of ambient microbial populations to the addition of selected electron donors for detritus and 0-10 cm soil (Table 4.1). Electron donors included various amino acids, carboxylic acids, alcohols, and polysaccharides added at a rate 0.83 mmole C g^{-1} dry soil. Preliminary experiments showed that C added at this rate did not limit heterotrophic microbial activity during a 2 d incubation (data not shown). All electron donors were added on a C-equivalent basis.

Anaerobic incubations were carried out for 2 d and CO_2 production was quantified at 4, 24, and 48 hr. Schott media bottles with screw-type lids containing approximately 10 g soil were sealed under a N_2 headspace to facilitate anaerobic conditions. Prior to the start of incubations, various electron donors were added to detritus and soil. After bottles were purged with N_2, incubations were carried out at approximate field soil-temperature conditions (30°C).

Carbon dioxide production was quantified using an alkali trap method in which 3 mL of 0.2 *M* NaOH was added to small vials placed inside incubation bottles. After 4, 24 and 48 hr of incubation, vials containing the NaOH traps were removed and capped. The CO_2 trapped was determined by gas chromatography after acidification of NaOH. To the enclosed NaOH in vials, excess HCl was added through a septum to neutralize NaOH and result in pH<2.0. A portion of the resulting CO_2 in headspace was sampled by syringe for injection into a gas chromatograph. Carbon dioxide was measured using a Shimadzu

Table 4.1. List of electron donors or nutrients added to detritus and soil for measurement of heterotrophic microbial activity for three incubations.

	March 1999		June 2001
Treatment	2 d	14 d	14 d
Alcohols	glycerol mannitol	glycerol mannitol	glycerol mannitol
Amides		glucuronamide	glucuronamide
Amino Acids	alanine cysteine aspartic acid glutamic acid histidine lysine methionine proline tyrosine	alanine cysteine aspartate glutamine	alanine cysteine
Aromatics		inosine	inosine
Carboxylic Acids	acetic formic oxalic butyric maleic propionic valeric		acetic formic oxalic
Plant Matter		*Typha* *Cladium*	*Typha* *Cladium*
Polysaccharides	glucose maltose	glucose maltose	glucose maltose
Nutrients			phosphate NH_4-N NH_4-N + phosphate

GC-8A gas chromatograph fitted with a thermal conductivity detector and a Poropak N column (Supelco, Bellefonte, PA). Injector/detector temperature was 30°C and column temperature was 25°C. The gas pressure in vials was measured using a digital pressure meter (Kane-May, Great Britain) along with solution pH. Carbon dioxide trapped in the NaOH was then calculated using a modification of a method described by (Martens, 1987) in which the universal gas law, pressure in the bottles, solution pH, CO_2 concentration in headspace (from the gas chromatograph), and solution and headspace volumes were used to calculate trapped CO_2. Appropriate controls containing no soil were included to account for background CO_2 contamination.

Impacts of Electron Donors on Heterotrophic Microbial Activity

This experiment involved the incubation of detritus and soil with various types of electron donors (Table 4.1), and subsequent measurement of anaerobic CO_2 and CH_4 production over a 14 d incubation period. Carbon dioxide production was determined using the same methods and analyses as for the short-term 2 d study, but with trap removal at 4 and 24 h and then at approximately 2 d intervals up to 14 d. Electron donors rapidly utilized by soil microorganisms (as indicated by CO_2 production rates) in the short-term study were selected for this study. In addition, several other electron donors including glucuronamides, aromatic compounds, and *Typha* and *Cladium* plant tissue were included. Electron donors were added at a predetermined rate so as to prevent C limitation during incubation (2.1 mmole C g^{-1} soil). Basal CO_2 production was also measured in the absence of any added electron donors. After addition of soil and electron donors to the bottles followed by N_2 purging, anaerobic incubations were carried out at a temperature of 30°C.

Methane production rates were measured in separate incubations but with the same electron donors and nutrients (Table 4.1). Approximately 10 g of detritus or soil were placed into serum bottles capped with butyl rubber septa and aluminum crimps. Samples were purged with N_2 to facilitate anaerobic conditions, and were then purged again every 4 d. Methane accumulation in headspace was monitored by sample headspace injection every 2 d to a gas chromatograph (Shimadzu GC-8A, Carboxyn 1000 column at 110°C, flame ionization detector at 160°C). Methane production rates were calculated using CH_4 concentrations and internal headspace pressure and volume.

Impacts of Selected Electron Donors and Nutrients on Heterotrophic Microbial Activity

Results from the previous short-term and 14 d incubations provided information regarding which electron donors were most readily utilized by heterotrophic microorganisms. Several additional treatments were included such as additions of inorganic N and P. Many soil physico-chemical properties were also quantified and related to heterotrophic microbial activities.

This experiment involved the incubation of 0-10 cm soil from the P-impacted and P-unimpacted reference areas with various electron donors and nutrients (Table 4.1), followed by subsequent measurement of anaerobic CO_2 and CH_4 production over a 14 d incubation period. The incubation and measurements of CO_2 and CH_4 production were the same as previously described using a 14 d incubation.

Methods of Analysis

In the laboratory, samples were weighed and homogenized thoroughly with 20 g subsamples then being dried at 70°C for 3 d to determine the percentage of solids and

water contents. Soil bulk density was then calculated for soil on a dry-weight basis. Total C contents of detritus and soil were determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Loss on ignition (LOI) was determined as the mass loss of soil after ashing at 550°C divided by the initial sample mass.

Soil total P (TP) was determined using an ashing method (Anderson, 1976). Approximately 0.2 g oven-dried soil was placed in a muffle furnace for 3-4 hr at 550°C. Samples were then acidified using 20 mL of 6 *M* HCl and digested for several hours until dry. Samples were then re-dissolved in 2.25 mL of 6 *M* HCl, filtered through Whatman #41 filter paper, brought to a final volume of 50 mL, and TP was then determined using an automated colorimetric procedure (U. S. EPA, 1993c). The $NaHCO_3$-P*i* was determined by extracting 10 g soil with 20 mL of 0.5 *M* $NaHCO_3$, shaking for 16 hr, filtering through Whatman #41 filter paper, and analyzing the extracts colorimetrically (U. S. EPA, 1993c).

Microbial biomass C (MBC) was determined by a fumigation-extraction method (Vance et al., 1987). Extractable total organic carbon (TOC) was determined by extracting soil with 20 mL of 0.5 *M* K_2SO_4, by shaking for 1 hr, and then filtering through Whatman #42 filter paper. The TOC was then measured using a Dohrmann total organic C analyzer (Rosemount Analytical, Santa Clara, CA).

Data Analysis

Comparisons of means of treatments were determined using Analysis of Variance (ANOVA) with Fisher's LSD at $P<0.05$. A completely randomized experimental design was utilized, with factors including electron donor treatments, field sampling stations,

and soil depth. Statistical differences at $P<0.05$ were compared between the P-impacted, transitional, and unimpacted areas. Electron donors were grouped based on their functional groups and comparisons were made between functional groups. The CO_2 and CH_4 production rates were determined by calculating the slope of CO_2 or CH_4 production over an incubation period of either 2 or 14 d. Turnover rates of added electron donors were calculated by dividing the sum of substrate-induced CO_2-C and CH_4-C-production rates by quantity of C addition to the soil.

Results

Short-Term Response of Ambient Soil Microbial Community

Soil characterization data are presented in Table 4.2. Total P and $NaHCO_3$-P*i* were highest in the P-impacted area and lowest at the reference area for both detritus and soil. Various C-related parameters were generally not influenced by P loading in detritus, but were higher in the P-impacted area in the 0-10 cm soil layer.

Basal CO_2 production was significantly highest in the P-impacted area and decreased at the reference area in detritus but not in soil (Table 4.3). Detritus CO_2 production was higher than in the 0-10 cm soil layer in P-impacted areas but not in the reference areas. The utilization of all electron donors was highest in the P-impacted area and lowest in the transitional and references areas in detritus. Addition of electron donors enhanced CO_2 production rates in 0-10 cm soil at all three sites along the P gradient. However, basal CO_2 production rates were not impacted by nutrient loading in the 0-10 cm soil. The polysaccharides provoked greatest enhancement of CO_2 production among all electron donors added in the P-impacted area in detritus. However, there were only a few differences in CO_2 production rates between electron-donor treatments for

Table 4.2. Soil characterization data for detritus and 0-10 cm soil at P-impacted, transitional, and reference areas of Water Conservation Area-2a (March 1999), with standard error values (n=3) (Chua, 2000).

Parameter	Units	P-Impacted	Transitional	Reference
Detritus				
Loss on Ignition*	%	88 -	85 -	82 -
Total C*	g C kg^{-1}	439 -	419 -	412 -
Total P	mg P kg^{-1}	1890 (21)	1050 (32)	693 (154)
$NaHCO_3$-P*i*	mg P kg^{-1}	14.8 (1.1)	0.0 (0.3)	0.0 (0.0)
Extractable Organic C*	mg C kg^{-1}	3403 -	3028 -	3183 -
Microbial Biomass C*	mg C kg^{-1}	3620 -	8758 -	4331 -
0-10 cm Soil				
Bulk Density	g cm^{-3}	0.09 (0.01)	0.10 (0.01)	0.09 (0.01)
Loss on Ignition*	%	89 -	88 -	85 -
Total C*	g C kg^{-1}	461 -	443 -	437 -
Total P	mg P kg^{-1}	796 (166)	688 (100)	336 (46)
$NaHCO_3$-P*i*	mg P kg^{-1}	2.9 (0.1)	2.0 (0.7)	0.2 (0.3)
Extractable Organic C*	mg C kg^{-1}	3900 -	2580 -	2290 -
Microbial Biomass C*	mg C kg^{-1}	2680 -	2530 -	733 -

* data from Wright et al., 2001c.

Table 4.3. The CO_2-production rates in detritus and 0-10 cm soil over a 2 d incubation for the P-impacted, transitional, and unimpacted reference areas of Water Conservation Area-2a (March 1999). Values followed by the same letter are not significantly different at $P<0.05$.

Treatment	P-Impacted	Transitional	Reference
Detritus	---------ug CO_2-C g^{-1} hr^{-1} ---------		
Basal	19 a	11 a	11 a
Alcohols	33 b	14 b	17 b
Amino Acids	33 b	15 b	17 b
Carboxylic Acids	33 b	21 c	24 b
Polysaccharides	42 c	20 c	19 b
0-10 cm Soil			
Basal	8 a	12 a	9 a
Alcohols	22 c	26 b	22 b
Amino Acids	21 c	21 b	21 b
Carboxylic Acids	23 c	23 b	22 b
Polysaccharides	18 b	22 b	26 c

both detritus and soil (Table 4.3). Additions of both C and N sources, as for the amino acid treatments, produced no significant response beyond that of treatments receiving only the C substrate.

Impacts of Electron Donors on Heterotrophic Microbial Activity

Only the P-impacted and reference areas were used in the long-term incubations. The CO_2-production rates were significantly higher in the P-impacted area than in the reference area for basal respiration and for all electron donor treatments in the detritus (Table 4.4). In detritus, treatments receiving polysaccharides produced significantly higher CO_2 concentrations than other treatments. Plant matter showed the least enhancement of CO_2-production rates of all treatments in both detritus and soil. The CO_2-production rates in detritus were significantly higher than in 0-10 cm soil. Few impacts of nutrient loading were observed in 0-10 cm soil, with CO_2-production rates being similar at various sites along the P gradient. In soil, only the inosine and polysaccharide treatments had significantly higher CO_2-production rates in the impacted area than the reference area.

Basal CH_4-production rates were not influenced by P loading in either the detritus or 0-10 cm soil (Table 4.4). The addition of plant matter enhanced CH_4 production the most in P-impacted detritus, while addition of polysaccharides and alcohols did not stimulate CH_4 production in the P-impacted area. In fact, detrital CO_2-production rates in the reference area were significantly higher than in the P-impacted area when polysaccharides and alcohols were added. In soil, only treatments receiving plant matter and aromatics resulted in higher CO_2-production rates in the P-impacted area than the reference area. Methane production rates were significantly greater in detritus than in

Table 4.4. The CO_2 and CH_4-production rates over a 14 d incubation in detritus and 0-10 cm soil at the P-impacted and reference areas of Water Conservation Area-2a (March 1999). Statistical differences between the P-impacted and reference areas at $P<0.05$ are denoted by significant (S) and not significant (NS). Values followed by the same letter are not significantly different at $P<0.05$.

Treatment	P-Impacted	Reference	($P<0.05$)	P-Impacted	Reference	($P<0.05$)
Detritus	---ug CO_2-C g^{-1} d^{-1} ---			---ug CH_4-C g^{-1} d^{-1} ---		
Basal	254 a	187 a	S	159 a	228 ab	NS
Alcohols	521 cd	287 b	S	156 a	365 c	S
Amides	519 cd	311 bc	S	739 c	271 b	S
Amino Acids	537 cd	355 bc	S	295 b	254 ab	NS
Aromatics	530 cd	293 b	S	316 b	157 a	S
Plant Matter	348 b	202 a	S	782 c	273 b	S
Polysaccharides	655 d	370 c	S	143 a	391 c	S
0-10 cm Soil						
Basal	43 a	50 a	NS	5 a	13 a	NS
Alcohols	183 b	96 b	NS	104 bc	61 b	NS
Amides	196 b	151 bc	NS	90 b	85 b	NS
Amino Acids	231 bc	216 cd	NS	70 b	57 b	NS
Aromatics	247 bc	153 bc	S	61 b	27 a	S
Plant Matter	148 b	134 bc	NS	129 c	75 b	S
Polysaccharides	282 c	115 b	S	63 b	56 b	NS

underlying 0-10 cm soil. In fact, basal CH_4 production rates in 0-10 cm soil were only 3-6% of basal rates in detritus.

In both the P-impacted and reference area, anaerobic CO_2-production rates were several orders of magnitude greater than the CH_4-production rates. Subtraction of basal rates from treatment rates yielded values for substrate induced respiration. In the P-impacted area, polysaccharides were most readily utilized in both detritus and soil while alcohols and polysaccharides were preferred by microorganisms in the reference areas. Meanwhile, plant matter was utilized least by CO_2-producing microorganisms in both areas. For CH_4 production, plant matter was the most utilized electron donor in the P-impacted area in both detritus and soil.

Impacts of Selected Electron Donors and Nutrients on Heterotrophic Microbial Activity

Soil characterization data are presented in Table 4.5. Soil P parameters were significantly higher in the P-impacted area than the reference area. However, microbial biomass C was significantly higher in the reference area than in the P-impacted area. The soil from the P-impacted area consisted of consolidated, decomposed plant detritus while soil from the P unimpacted or reference areas consisted of consolidated plant detritus with large amounts of calcareous, benthic periphyton or algal mats due to low water levels during sampling. The periphyton consisted of an assemblage of benthic or floating algae with associated microbial communities, while soil from the P-impacted area consisted primarily of older, more consolidated *Typha* detrital matter devoid of periphyton. Basal CO_2 and CH_4 production rates were higher in the reference area than the P-impacted area, which was consistent with microbial biomass differences (Tables 4.5 and 4.6). Addition of NH_4-N, P, or NH_4-N + P did not impact CO_2 or CH_4-

Table 4.5. Characterization data for 0-10 cm soil at the F1 (P-impacted) and U3 (reference) areas of Water Conservation Area-2a (June 2001) with standard error values (n = 3).

Parameter	Units	P-Impacted	Reference
Bulk Density	$g\ cm^{-3}$	0.10 (0.0)	0.07 (0.0)
Loss on Ignition	%	80 (2)	69 (1)
Total C	$g\ C\ kg^{-1}$	409 (6)	343 (3)
Total P	$mg\ P\ kg^{-1}$	1090 (119)	426 (23)
$NaHCO_3$-P*i*	$mg\ P\ kg^{-1}$	77 (17)	2 (0.3)
Extractable Organic C	$mg\ C\ kg^{-1}$	2800 (161)	2370 (139)
Microbial Biomass C	$mg\ C\ kg^{-1}$	4040 (578)	16900 (215)

Table 4.6. The CO_2 and CH_4-production rates in 0-10 cm soil over a 14 d incubation in F1 (P-impacted) and unimpacted U3 (reference) areas of Water Conservation Area-2a. Statistical differences between the P-impacted and reference areas at $P<0.05$ are denoted by significant (S) and not significant (NS). Values followed by the same letter are not significantly different at $P<0.05$.

Treatment	P-Impacted	Reference	$P<0.05$
CO_2 Production	----ug CO_2-C g^{-1} d^{-1} ----		
Basal	84 a	222 a	S
Nutrients	85 a	289 a	S
Alcohols	313 c	331 ab	NS
Amides	494 e	575 c	NS
Amino Acids	383 d	255 a	S
Aromatics	379 d	309 a	NS
Carboxylic Acids	290 c	396 bc	NS
Plant Matter	155 b	187 a	NS
Polysaccharides	375 d	187 a	S
CH_4 Production	----ug CH_4-C g^{-1} d^{-1} ----		
Basal	22 a	80 b	S
Nutrients	21 a	117 bc	S
Alcohols	96 b	214 d	S
Amides	260 d	228 d	NS
Amino Acids	136 bc	102 bc	NS
Aromatics	158 bc	118 c	S
Carboxylic Acids	111 b	30 a	S
Plant Matter	170 c	141 c	NS
Polysaccharides	169 c	216 d	S

production rates in the P-impacted area (data not shown), however, both CO_2 and CH_4-production rates were enhanced by addition of inorganic nutrients in the reference area.

The addition of various electron donors produced mixed responses (Table 4.6). Addition of all electron donors enhanced CO_2-production rates in the P-impacted area while only amides and carboxylic acids enhanced rates in the reference area. Similar trends were observed in terms of CH_4 production, with all electron donors enhancing rates at the P-impacted area but only a few donors enhancing rates at the reference area. When basal CO_2-production rates were subtracted, the enhancement of CO_2 production by electron donors was generally greater in the P-impacted area than the reference area. Amide addition significantly enhanced both CO_2 and CH_4-production rates more than addition of other donors. Similar to CO_2 production, substrate-induced enhancement of CH_4 production above basal rates was generally higher in the P-impacted area than the reference area (Table 4.6). Addition of C sources enhanced CH_4-production rates in both the P-impacted and reference areas, with the exception of carboxylic acids at the reference area.

Methane production rates were approximately 45% of CO_2 production rates at both P-impacted and reference areas. The contribution of CH_4 production to overall CO_2 + CH_4 production was similar for all electron donors except plant matter. Addition of plant matter resulted in CH_4 contributions from 75 to 109% of the CO_2-production rates for the reference and P-impacted areas, respectively.

Turnover rates of added electron donors were determined by dividing CO_2 + CH_4 production rates by the amount of C added (25.2 mg C g^{-1} soil). Data from both 14 d experiments were combined into Table 4.7. Turnover rates were significantly higher in

Table 4.7. Turnover rates (d^{-1}) of various electron donors in detritus and soil. Values followed by the same letter are not significantly different at $P<0.05$. Turnover rates are the sum of CO_2 and CH_4-production rates divided by C added (ug C g^{-1}).

Electron Donor	n	P-Impacted	Reference
Detritus		----------d^{-1}----------	
Alcohols	6	0.011 a	0.009 b
Amides	3	0.034 c	0.007 ab
Amino Acids	12	0.017 a	0.008 ab
Aromatics	3	0.017 a	0.004 a
Plant Matter	6	0.028 bc	0.002 a
Polysaccharides	6	0.016 a	0.014 c
0-10 cm Soil			
Alcohols	12	0.011 ab	0.007 ab
Amides	6	0.018 c	0.013 bc
Amino Acids	18	0.013 ab	0.005 a
Aromatics	6	0.014 ab	0.005 a
Carboxylic Acids	9	0.006 a	0.003 a
Plant Matter	12	0.009 a	0.004 a
Polysaccharides	12	0.015 bc	0.005 a

the P-impacted area than in the reference area in both detritus and soil (Table 4.7). Turnover rates were also significantly higher in detritus than in 0-10 cm soil. In detritus, turnover rates for amides, plant matter, and polysaccharides treatments were generally higher than for other electron donors. In 0-10 cm soil, turnover rates of amides were highest, while carboxylic acids and plant matter turnover rates were generally lower.

Discussion

Short-term incubations measure the ability of soil microorganisms to rapidly utilize certain substrates, which suggests the presence of enzyme systems capable of their utilization (Degens and Harris, 1997; Degens, 1998). This in turn suggests a previous exposure to certain substrates and provides an indication of what types of electron donors are commonly present in soils. All added electron donors contributed to enhanced CO_2 production during the 2 d incubation in both detritus and soil, suggesting that a wide range of electron donors are commonly present and being utilized in Everglades soil.

Detritus and soil along the WCA-2a nutrient gradients typically contained over 2000 mg extractable TOC kg^{-1} (Tables 4.2 and 4.5). The high utilization rates of electron donors suggested that heterotrophic microbial activity was limited by available organic C in detritus and soil. Even though extractable TOC concentrations were high, much of the soil-solution C may not be available to soil microorganisms. Thus, carbon limitation to microbial activity seemed to be observed both in the P-impacted and reference areas of WCA-2a. Enhancement of CO_2 and CH_4-production rates by added electron donors has also been observed in Everglades soils and marl (Bachoon and Jones, 1992; Amador and Jones,1993; Wright and Reddy, 2001b).

Turnover rates of added electron donors showed that amides, plant matter, and polysaccharides were readily utilized by soil microorganisms in detritus (Table 4.7). Turnover rates of amides were also higher than for most other electron donors in 0-10 cm soil, but exhibited greater variability. Utilization rates of electron donors were generally greater in the P-impacted area. Higher nutrient loading in the P-impacted area generally supported higher microbial biomass and higher concentrations of limiting nutrients (DeBusk, 1996; Drake et al., 1996; White and Reddy, 2000). Heterotrophic microbial activity in the unimpacted reference area of WCA-2a appeared to be limited by inorganic N and P in addition to labile organic C. Additions of NH_4-N, P, or NH_4-N + P did not impact CO_2 or CH_4-production rates in the P-impacted area, however, both CO_2 and CH_4-production rates were increased by nutrient addition at the reference area. Increased nutrient availability often enhances heterotrophic microbial activity in soils (Webster and Benfield, 1986; Bridgham and Richardson, 1992; DeBusk, 1996). Similarly, P additions had no impact on heterotrophic microbial activity in high-P soils in other studies (Amador and Jones, 1995). This was expected since P was the limiting nutrient for microorganisms in P-unimpacted areas of WCA-2a (McCormick et al., 2000, Wright and Reddy, 2001a,b). Additions of electron donors to the P-limited reference area likely resulted in immobilization of available nutrients, thus further limiting CO_2 and CH_4-production rates.

Basal CO_2-production rates were generally highest in P-impacted areas than in reference areas as observed during March 1999. Basal and substrate-induced CO_2-production rates have been shown to be higher in P-impacted soils than in unimpacted soils (Amador and Jones, 1995; DeBusk, 1996; Wright and Reddy, 2001b).

Enhancement of basal CO_2 production rates in the P-impacted areas compared to the reference area during March 1999 can be explained by higher P concentrations in the P-impacted area. In addition, P loading along the WCA-2a P gradient has been shown to enhance microbial biomass C, N, and P (DeBusk and Reddy, 1998; White and Reddy, 2000). In detritus, enhanced microbial biomass in addition to nutrient loading resulted in increased basal CO_2-production rates at the P-impacted area compared to the P-limited reference area.

During June 2001, the reference area soil contained large amounts of biologically-active detrital periphyton; thus, microbial biomass was higher in these soils than in the P-impacted area (Table 4.5). This had major influence on the CO_2 and CH_4-production rates, whereas basal rates were higher in reference areas than in P-impacted areas. This was in contrast to other studies that showed enhanced CO_2 and CH_4 production in the P-impacted area compared to the reference area (DeBusk and Reddy, 1998; Wright and Reddy, 2001b). However, in these other studies, soil from the reference area did not contain large amounts of detrital periphyton as did the June 2001 samples. Thus, not only did P loading have a significant influence on CO_2 and CH_4 production rates, but microbial biomass was also an important factor regulating heterotrophic microbial activities in Everglades soil. Indeed, significant correlations between microbial biomass and heterotrophic microbial activity have been observed in several studies (DeBusk and Reddy, 1998; White and Reddy, 2000; Wright and Reddy, 2001b).

The CO_2 and CH_4-production rates were significantly higher in surface detritus than in underlying soil. Decreases of CO_2 and CH_4 production with soil depth in Everglades soils are commonly observed (Bachoon and Jones, 1992; DeBusk and Reddy,

1998; Wright and Reddy, 2001b). Wetland soils exhibit an oxidized layer at the soil-water interface, and such aerobic conditions may facilitate enhanced microbial biomass in surface detritus compared to the underlying 0-10 cm soil (Reddy and D'Angelo, 1994). This enhanced biomass may result in increased heterotrophic microbial activity in detritus compared to underlying soil. This also could be due to higher substrate quality in the less-decomposed surface detritus compared to underlying soil (DeBusk and Reddy, 1998).

The addition of electron donors to soil often results in enhanced microbial biomass, with addition of glucose to soil tripling microbial biomass within 5 d (Nannipierri et al., 1979). In Everglades soils, addition of various electron donors likely enhanced microbial biomass during the incubation period. Thus, much of the added soluble C may have been assimilated into biomass rather than released as CO_2 via respiratory pathways or fermentation. However, plant matter was not as readily available as the other soluble electron donors; thus, plant matter may not have significantly enhanced microbial biomass during the incubation period, and most C from plant matter degradation was released as CO_2 rather than assimilated into microbial biomass. Thus, the efficiency of C utilization was higher when plant matter was added compared to other electron donors.

In both the P-impacted and the reference area, anaerobic CO_2-production rates were significantly higher than CH_4-production rates. The CH_4-production rates are often lower than anaerobic CO_2-production rates, because CO_2 production also occurs via other processes in wetland soils such as denitrification, SO_4 reduction, and fermentation (Lovley and Klug, 1986; D'Angelo and Reddy, 1999; Wright and Reddy, 2001b). Plant

matter showed the least enhancement of CO_2 production rates of all electron-donor treatments in detritus and soil. Degradation of plant material requires the presence of various extracellular enzymes to degrade large particulates to smaller molecules that can then be taken up by microbial cells (Chrost, 1991). The limited surface area of added plant matter may have also depressed its availability to anaerobic CO_2-producing microorganisms (Heal et al., 1981). *Typha* and *Cladium* plant matter was not added in soluble form like other electron donors, so lower CO_2 production rates were expected.

The external loading of electron acceptors in inflow waters, such as nitrate (NO_3) and sulfate (SO_4), to the P-impacted area (McCormick et al., 2000), likely supported denitrification and SO_4 reduction at the expense of methanogenesis. This may explain higher CH_4-production rates in the reference area compared to the P-impacted area, which is less impacted by nutrient loading.

Various carboxylic acids, such as formate and acetate, are often precursers for CH_4 production and can stimulate methanogenesis (Lovley and Klug, 1986; Drake et al., 1996). However, considerable SO_4 concentrations have been observed in WCA-2a soil (Schipper and Reddy, 1994) which may have enhanced the ability of SO_4-reducing microorganisms to out-compete methanogens for these substrates (Drake et al., 1996). Methane production rates were approximately 45% of CO_2-production rates. In other studies of Everglades soil, CH_4 production contributed less than one-third of anaerobic CO_2-production rates (DeBusk, 1996; Wright and Reddy, 2001b). Methanogens are often out-competed for electron donors by SO_4-reducing microorganisms due to differences in thermodynamic energy yields (Achtnich et al., 1995). In this study, addition of electron donors likely enhanced microbial biomass and C assimilation, leading to rapid

consumption of NO_3 and SO_4, and thus to enhanced methanogenesis while retarding denitrification and SO_4 reduction.

Conclusions

Soil microorganisms along the nutrient gradient in WCA-2a showed the capability to degrade a wide range of electron donors under varying levels of P enrichment. Addition of various electron donors enhanced both anaerobic CO_2 and CH_4-production rates in detritus and in underlying 0-10 cm soil. The rapid microbial utilization of electron donors suggested that heterotrophic microbial activity was limited by labile organic C. Inorganic nutrients such, as NH_4-N and P, limited heterotrophic microbial activity only in the P-unimpacted reference area in the interior of WCA-2a. The CO_2 and CH_4-production rates were highest in surface detritus and decreased with soil depth. Microbial biomass appeared to be a major factor controlling basal CO_2 and CH_4 production in addition to nutrient loading. Thus, heterotrophic microbial activity was dependent on the initial soil microbial biomass and was limited by available C and by inorganic NH_4-N and P. Continued external nutrient loading into the P-unimpacted interior areas of WCA-2a may further enhance organic matter degradation and nutrient regeneration from soil.

CHAPTER 5
LONG-TERM NUTRIENT LOADING IMPACTS ON MICROBIAL PROCESSES IN SELECTED HYDROLOGIC UNITS OF THE EVERGLADES

Introduction

Historically, the Florida Everglades wetland ecosystems developed as relatively nutrient-poor and supported vegetation adapted to such conditions (Davis, 1943). During the past century, the Everglades has been drained, divided by levees and canals, and separated into distinct hydrologic units where water movement and storage is regulated. These units include the Everglades Agricultural Area (EAA), Water Conservation Area-1 (WCA-1), WCA-2a, and WCA-3a, and the Everglades National Park (ENP). Nutrient runoff from the EAA in addition to altered hydrologic conditions has been implicated in altering the Everglades wetland systems by increasing soil nutrient levels (particularly P) which has promoted subsequent vegetation intrusions by *Typha* into areas previously dominated by *Cladium*, such as observed in northern sections of WCA-2a (Davis, 1991; Koch and Reddy, 1992; DeBusk et al., 1994; DeBusk et al., 2001a). In addition, the impact of anthropogenic nutrient loading to the Everglades is documented in the distribution of floodwater and soil total P (DeBusk et al., 1994; Newman et al., 1997; Reddy et al., 1998). The hydrologic units WCA-1, WCA-2a, WCA-3a, and Taylor Slough (TS) from the ENP differ in varying degrees in their vegetation composition and also in soil total phosphorus (TP) concentrations (McCormick et al., 2000).

Impacts of phosphorus (P) loading on soil chemical and microbial processes have been observed in many studies (Amador and Jones, 1993; Newman et al., 1997; Reddy et al., 1998; White and Reddy, 1999; Wright and Reddy, 2001a,b). Addition of limiting nutrients to ecosystems often results in enhanced productivity of vegetation and stimulation of the microbial community (Chrost, 1991; Craft and Richardson, 1995; Chiang et al., 2000; Newman et al., 2001). However, microbial communities may be more sensitive to or respond more readily to increases in nutrient levels than do vegetational communities. Changes in vegetation patterns due to nutrient loading may take years to be observed (Chiang et al., 2000; McCormick et al., 2000), while microbial processes may be altered after only a short exposure to increased nutrient levels (McCormick and O'Dell, 1996).

Nutrient loading into oligotrophic systems often results in increased microbial biomass, which in turn can be responsible for enhanced organic matter degradation and heterotrophic microbial activity (Martens, 1995; White and Reddy 2000; Wright and Reddy, 2001b). An understanding of the impacts of nutrient loading on microbial processes and on relationships between nutrients, microbial biomass, and related microbial processes is important, because organic matter degradation and nutrient cycling are dependent on the chemical and physical composition of organic matter, microbial community composition, and availability of nutrients (Webster and Benfield, 1986; Martens, 1995; Rybczyk et al., 1996). Microbial biomass is considered to be the key component regulating organic matter degradation and nutrient cycling in soil (Wardle, 1992; Martens, 1995).

In addition to contributing to changes in vegetation patterns of the Everglades (Craft and Richardson, 1995; Richardson et al., 1997; Miao and Sklar, 1998), P loading has altered various soil chemical and microbial processes, both stimulatory and negative to some degree (Amador and Jones, 1993; McCormick and O'Dell, 1996; Newman et al., 1997; Reddy et al., 1998; Wright and Reddy, 2001a,b). Microbial biomass, heterotrophic microbial activity, and various enzyme activities have been enhanced or depressed at P-impacted sites of various hydrologic units (Amador and Jones, 1993; DeBusk and Reddy, 1998; White and Reddy, 1999; Wright and Reddy, 2001a,b). However, such changes in microbial community dynamics may also be regulated in part by variables such as vegetation or hydrologic conditions (McCormick et al., 2000).

The objectives of this research were to: 1) determine impacts of nutrient loading on soil biogeochemical indicators as a function of distance from primary water inflow points in four selected hydrologic units of the Everglades; 2) determine relationships between various soil biogeochemical indicators as a function of soil physico-chemical properties; and 3) develop soil chemical and microbial indicators of nutrient enrichment.

Materials and Methods

Site Description

A diagram showing the locations of WCA-1, WCA-2a, WCA-3a, and TS hydrologic units of the Everglades ecosystem is shown in Fig. 5.1. The WCA-1 is a national wildlife refuge encompassing 59,000 ha of the northern Everglades wetland ecosystem. Rainfall is the primary water input into WCA-1, with remaining sources of water include P-laden runoff from the EAA. Most of the increased soil TP levels have been observed in areas adjacent to canals or levees (McCormick et al., 2000). *Typha*

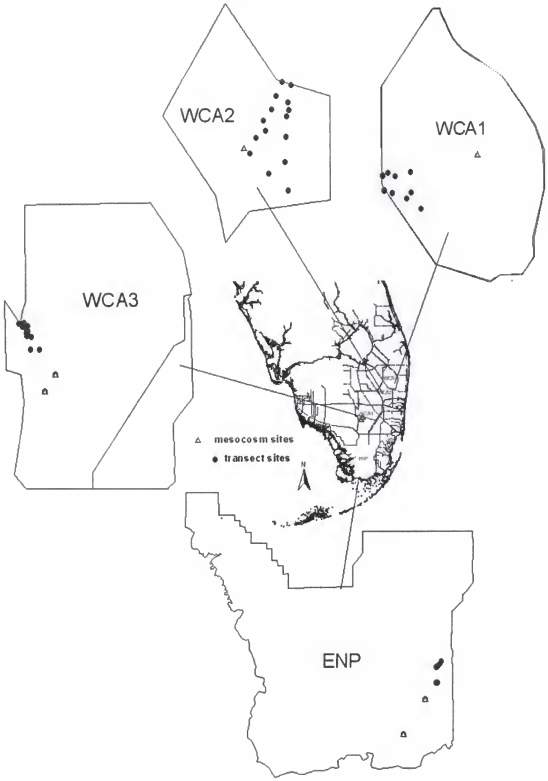

Figure 5.1. Locations of experimental sites along nutrient gradients in Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough of Everglades National Park (ENP).

predominates in P-impacted areas while *Cladium*, open sloughs, and tree islands are common in unimpacted, interior areas. Two soil TP gradients encompassing various soil TP concentrations were observed in the southwest section of WCA-1 from the S-6 pump station at the western margin of the hydrologic unit to the interior of WCA-1.

The WCA-2a (44,700 ha) receives drainage water from WCA-1 in addition to discharge water from the EAA. The impact zone of WCA-2a is much broader and extends much farther into the interior than the impact zone in WCA-1, thus P impacts on vegetation community structure are more evident in WCA-2a (McCormick et al., 2000). The WCA-2a vegetation consists of *Typha* in P-impacted areas, while *Cladium* and sloughs dominate in the interior of WCA-2a. Three transects were established in WCA-2a, designated the E, F, and U transects. The E and F transects extend from the S10-C water inflow structure in a southern direction into the interior of WCA-2a. These transects extend from the P-impacted, *Typha*-dominated areas near S10-C to P unimpacted, *Cladium*-dominated areas in the interior of WCA-2a. The U transects are located in the unimpacted interior of WCA-2a.

The WCA-3a also receives drainage water from northern areas, particularly WCA-2a, but rainfall contributes approximately 42% of its annual water budget (Reddy et al., 1998). In WCA-3a, various sites were selected up to 6 km east and west of the primary water inflow canal. In WCA-3a, tree islands and wet prairies comprise the vegetation community structure, while most of the sites near the canal have been impacted by P loading. In addition, two sites were selected in the interior of WCA-3a in P-unimpacted areas.

Taylor Slough is located south of WCA-3a and serves as a bridge with Florida Bay, and receives discharge water from WCA-3a. The soil of TS differs from other hydrologic units as wet prairies formed on marl sediments under P limited conditions. Sites were selected along two transects encompassing various soil TP concentrations from the primary water inflow point. Additional sites were selected in unimpacted areas to assess background levels of biogeochemical indicators.

A listing of sites within the four hydrologic units and their distances from the primary water inflow points is presented in Table 5.1.

Soil Sampling

Soil samples were collected by driving a 10 cm diameter corer into soil to a depth of 20 cm. Floc consisted of benthic periphyton or algae suspended in the water column that was present on the soil surface. This material was poured from above the soil surface by inverting the cores. The 0-3 cm soil layers were placed into plastic bags and stored at 4°C until analysis. The WCA-1 transects were sampled during May 1997, March 1998, and October 1998 while WCA-2a transects were sampled during April 1997, March 1998, and September 1998. The WCA-3a transects were sampled during March and November 2000, while TS transects were sampled during March and December 2000.

Methods of Analysis

Physico-Chemical Properties

In the laboratory, samples were weighed, homogenized thoroughly, and 20 g samples were dried at 70°C for 72 hr to determine the percent of solids and water content. Soil bulk density was then calculated on a dry-weight basis. Total carbon (C) and

Table 5.1. Station locations along various nutrient gradients in Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough with distances from primary water inflow points.

WCA-1	(km)	WCA-2a	(km)	WCA-3a	(km)	Taylor Slough	(km)
X 1	0.46	E1	2.27	E-05	0.59	05-E	0.30
X 2	1.25	E2	3.25	E-10	1.53	1-E	0.95
X 3	2.22	E3	4.19	E-15	2.36	15-E	1.74
X 4	4.37	E4	6.98	E-20	3.03	05-W	0.36
Y 4	3.23	E5	10.10	E-40	6.26	1-W	1.06
Z 1	0.26	F1	1.84	W-05	0.66	15-W	1.70
Z 2	1.08	F2	3.82	W-10	1.22	2-3	2.32
Z 3	2.24	F3	5.58	W-15	2.18	2-4	2.45
Z 4	3.12	F4	6.84	W-20	2.85	3-3	6.45
		F5	8.17	W-40	5.83	3-4	6.50
		U1	14.47	N-meso	12.88	N-Meso	11.98
		U2	12.56	S-meso	15.54	S-Meso	24.10
		U3	10.80				

nitrogen (N) content of floc and soil were determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Loss on ignition (LOI) was determined as the mass loss of soil after ashing at 550°C divided by the initial sub-sample mass.

Extractable ammonium (NH_4-N) was determined by shaking wet soil samples with 20 mL of 0.5 *M* K_2SO_4 for 1 hr on a longitudinal shaker. Samples were centrifuged for 10 min at 6000 rpm and vacuum-filtered through Whatman #42 filter paper. The supernatant was collected and was determined colorimetrically for NH_4-N (U. S. EPA, 1993b).

Soil TP was determined using an ashing method (Anderson, 1976). Approximately 0.2 g oven-dried soil was placed in a muffle furnace for 3-4 hr at 550°C. Samples were then acidified using 20 mL of 6 *M* HCl and digested for several hours until dry. Samples then were re-dissolved in 2.25 mL of 6 *M* HCl, filtered through Whatman #41 filter paper, brought to a final volume of 50 mL, and then TP was determined using an automated colorimetric procedure (U. S. EPA, 1993c). Soil total inorganic P (TP*i*) was determined by extracting 0.5 g dried, ground soil with 25 mL of 1.0 *M* HCL for 3 hr, followed by vacuum filtration using 0.45 µM membrane filters, and colorimetric analysis (U. S. EPA, 1993c) (Reddy et al., 1998).

Microbial Biomass

Microbial biomass C (MBC) was determined using the fumigation-extraction method of Vance et al. (1987). Approximately 5 g samples were placed into polypropylene centrifuge tubes, a 0.5 mL volume of chloroform was added, and tubes placed into a vacuum desiccater along with a beaker containing additional chloroform. A

non-fumigated control-sample set was placed on the adjacent lab bench. After 24 hr, samples were removed and both the controls (not exposed to chloroform) and the chloroform-treated samples were extracted with 20 mL of 0.5 *M* K_2SO_4, shaken for 1 hr on a longitudinal shaker, and vacuum filtered through #42 Whatman filter paper. The supernatant was collected and refrigerated at 4°C. Samples were analyzed for total organic carbon (TOC) using a Dohrmann total organic C analyzer (Rosemount Analytical, Santa Clara, CA). Microbial biomass C was determined by subtracting the extractable TOC in controls from the TOC for chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing a previous calibration for organic soils by Sparling et al. (1990). Extractable TOC was defined as the TOC from extracted, non-fumigated controls.

Microbial biomass N (MBN) was determined using the fumigation-extraction technique of Brookes et al., (1985). Ten mL of non-fumigated (control) and fumigated extracts from the MBC procedure were subjected to Kjeldahl-N digestion at 380°C for 4-5 hr using the salicylic acid modification of Bremner and Mulvaney (1982). Samples were brought to a final volume of 20 mL after digestion and transferred into scintillation vials. Extracts were analyzed for total Kjeldahl nitrogen (TKN) colorimetrically (U. S. EPA, 1993b). Microbial biomass N (MBN) was determined as the difference in TKN between fumigated and non-fumigated samples. A combined extraction efficiency and K*n* value of 0.54 was applied to values when calculating MBN (Brookes et al., 1985).

Microbial biomass P (MBP) was determined as the difference between TP of 0.5 *M* $NaHCO_3$ extracts of chloroform-fumigated and non-fumigated samples (Ivanoff et al., 1998). The MBP samples were incubated and fumigated as described for MBC. After a

24 hr incubation, both fumigated and non-fumigated samples were extracted with 20 mL of 0.5 *M* $NaHCO_3$ by shaking for 16 hr. After shaking, samples were centrifuged at 6000 rpm for 10 min and then filtered through 0.45 μM membrane filters into scintillation vials. Portions of the non-fumigated 0.5 *M* $NaHCO_3$ extracts were analyzed for P using an automated colorimeteric method (U. S. EPA, 1993c), with these results referred to as $NaHCO_3$-P*i* values. Other portions of both fumigated and non-fumigated 0.5 *M* $NaHCO_3$ extracts were digested for 4-5 hr at 380°C. The difference in TP between fumigated and non-fumigated digested samples is defined as MBP. No extraction efficiency factor was employed in the calculation of MBP.

Microbial Processes

Soil oxygen demand (SOD) was determined by measuring dissolved oxygen (O_2) depletion during a 24 hr incubation at 20°C. Approximately 2–5 g soil was added to 60 mL bottles followed by addition of O_2-saturated water to volume. The initial dissolved O_2 (DO) content of the slurry was then measured using a YSI Model 58 O_2 meter equipped with a DO probe (Yellow Springs, CO). After 24 hr incubation, samples were stirred and DO content measured. Soil oxygen demand was calculated as the difference between DO concentrations of O_2-saturated soil:water slurry at t = 0 minus the DO concentrations at 24 hr, multiplied by water volume and divided by sample mass.

Aerobic carbon dioxide (CO_2) production rates were determined in WCA-1 and WCA-2a mesocosms by measuring CO_2 production over a 10 d incubation period. Approximately 5-10 g wet samples were placed into glass bottles along with smaller vials containing 3 mL of a 0.5 *M* NaOH. Room air was used for aerobic incubations while bottles were purged with N_2 for anaerobic incubations. Vials containing the NaOH traps

were removed and capped at 2 d intervals up to 10 d. Later, 0.5 mL of 3 *M* HCl was added to the collected vials containing NaOH, and CO_2 in headspace was measured by gas chromatography (Shimadzu GC-8A, thermal conductivity detector at 25°C, Poropak N column at 20°C). Gas pressures in the vials were measured before gas sampling using a digital pressure meter (Kane-May, Great Britain) and was used in the calculation of CO_2 production. Appropriate controls containing no soil were included to account for background CO_2 concentrations.

Methane (CH_4) production rates were determined using 5-10 g wet samples placed into glass serum bottles sealed with butyl rubber septa and incubated at 30°C for 10 d. At time intervals of approximately 2 d, aliquots of headspace were sampled and run on a Shimadzu gas chromatograph-8A GC fitted with a flame ionization detector (160°C), and a Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 110°C. Gas pressure in the vials was measured before gas sampling using a digital pressure meter (Kane-May, Great Britain). Appropriate controls containing no soil were included to account for background CH_4 concentrations.

Potentially mineralizable N (PMN) was determined using anaerobic incubations over a 10 d period. Glass serum bottles were prepared by adding 10 g of wet samples and 5 mL of distilled water. Bottles were capped with butyl rubber septa, sealed with aluminum crimps, and the headspace filled with N_2 gas. Serum bottles were incubated in the dark at 40°C for 10 d. A set of samples was also prepared similarly but without incubation and served as time 0 controls. Samples were extracted with 20 mL of 0.5 *M* K_2SO_4 while shaking for 1 hr on a longitudinal shaker, and centrifuged for 10 min at 6000 rpm. The supernatant was filtered through Whatman #42 filter paper, collected in

scintillation vials, and analyzed for NH_4-N (U. S. EPA, 1993b). The difference between NH_4-N concentrations for samples incubated for 10 d and for time 0 controls was referred to as PMN.

Potentially mineralizable P (PMP) was determined using anaerobic incubations over a 10 d period. Incubations for PMP were carried out as described for the PMN determinations. The PMP samples were extracted with 20 mL of 1.0 *M* HCl, shaken for 3 hr, centrifuged for 10 min at 6000 rpm, and filtered through 0.45 μM membrane filters. Extracted P was then quantified colorimetrically (U. S. EPA, 1993c). The PMP was determined as the difference between P concentrations of extracts of the 10 d incubation and the time 0 control samples.

Data Analysis

Data were statistically analyzed using an analysis of variance (ANOVA) model to determine significant differences ($P<0.05$) between distances of sites from water inflow points, seasonality, soil depth, and for comparisons between the hydrological units. Treatment comparisons were based on a Fisher's LSD at $P<0.05$ using CoStat (Minneapolis, MN). Relationships between various chemical and microbial parameters were investigated using correlation coefficients (r) at $P<0.05$ to determine the most appropriate predictors of nutrient loading. Multiple regressions were also developed to identify significant factors that might explain various microbial processes. Respective sites within P-impacted and P-unimpacted areas were grouped for statistical comparisons. Delineations between areas designated as P-impacted or unimpacted within respective hydrologic units were based on significant differences in floc and 0-3 cm soil TP values as a function of distance from the primary water inflow points.

Results

Physico-Chemical Properties

Water content in WCA-1, WCA-2a, and WCA-3a floc was >95% but was significantly less (92%) in TS floc (data not shown). In 0-3 cm soil, water content in WCA-1, WCA-2a, and WCA-3a was approximately 90% but was only 83% in TS soil. Taylor Slough soil was more consolidated than other hydrologic units, resulting in lower water content. Bulk density of the 0-3 cm soil layer generally ranged from 0.05–0.10 g cm^{-3} for WCA-1, WCA-2a, and WCA-3a and 0.15-0.25 g cm^{-3} for TS (data not shown). Bulk density was not affected by P loading and was not variable over the sampling period.

Biogeochemical indicators in floc samples are presented in Tables 5.2 and 5.3, while indicators in 0-3 cm soil are presented in Tables 5.4 and 5.5. The LOI exhibited mixed response to P loading, with LOI generally being higher in P-impacted areas than unimpacted areas. No significant changes in LOI with depth were observed among the hydrologic units except for TS. However, LOI was significantly lower for TS than for LOI values for other hydrologic units, due to the calcareous nature of TS soil. In floc, extractable TOC showed an inverse relationship to P loading, with concentrations being lower in P-impacted areas near water inflow points. Extractable TOC concentrations were almost twice as high in floc as in underlying 0-3 cm soil. The WCA-3a had significantly higher extractable TOC concentrations than did other hydrologic units while TS exhibited the lowest concentrations.

In floc, extractable NH_4-N was generally higher in P-impacted areas than in unimpacted areas in WCA-1 and WCA-3a (Tables 5.2-5.5). In soil, NH_4-N

Table 5.2. Various floc parameters in P-impacted and unimpacted reference areas of Water Conservation Area-1 (WCA-1) and WCA-2a. Means averaged over the various seasonal sampling periods are presented. Significant differences between P-impacted and reference areas of the respective hydrologic units are noted.

Parameter	Units	WCA-1			WCA-2a		
		P-Impacted (n=6)	Reference (n=20)	(P<0.05)	P-Impacted (n=16)	Reference (n=12)	(P<0.05)
Loss on Ignition	%	84	88	NS	84	78	NS
Extractable Organic C	g C kg^{-1}	7	14	S	4	6	S
Extractable NH_4-N	mg N kg^{-1}	663	608	NS	81	117	S
$NaHCO_3$-*Pi*	mg P kg^{-1}	13	5	NS	50	2	S
Total P	mg P kg^{-1}	1670	619	S	1410	497	S
Total Inorganic P	mg P kg^{-1}	713	217	S	489	173	S
Total N	g N kg^{-1}	36	37	NS	30	28	NS
Total C	g C kg^{-1}	417	426	NS	420	393	NS
Microbial Biomass C	g C kg^{-1}	27	49	S	14	23	NS
Microbial Biomass N	mg N kg^{-1}	4700	6240	NS	1830	2600	NS
Microbial Biomass P	mg P kg^{-1}	662	401	NS	331	160	S
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	145	82	S	124	159	NS
Aerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	51	51	NS	13	15	NS
Anaerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	65	152	S	16	16	NS
PMN	mg N kg^{-1} d^{-1}	346	378	NS	78	92	NS
PMP	mg P kg^{-1} d^{-1}	45	11	S	14	6	S

NS = not significant at P<0.05; S = significant at P<0.05

Table 5.3. Various floc parameters in P-impacted and unimpacted reference areas of Water Conservation Area-3a (WCA-3a) and Taylor Slough. Means averaged over the various seasonal sampling periods are presented. Significant differences between P-impacted and reference areas of the respective hydrologic units are noted.

Parameter	Units	WCA-3a			Taylor Slough		
		P-Impacted (n=33)	Reference (n=30)	($P<0.05$)	P-Impacted (n=36)	Reference (n=12)	($P<0.05$)
Loss on Ignition	%	88	78	S	41	51	NS
Extractable Organic C	g C kg^{-1}	13	15	NS	6	6	NS
Extractable NH_4-N	mg N kg^{-1}	468	360	S	136	137	NS
$NaHCO_3$-P*i*	mg P kg^{-1}	146	5	S	1	1	NS
Total P	mg P kg^{-1}	1720	452	S	258	205	NS
Total Inorganic P	mg P kg^{-1}	683	186	S	104	81	S
Total N	g N kg^{-1}	39	35	S	13	32	S
Total C	g C kg^{-1}	439	409	S	244	374	S
Microbial Biomass C	g C kg^{-1}	45	72	S	15	21	NS
Microbial Biomass N	mg N kg^{-1}	4180	3330	NS	1060	1050	NS
Microbial Biomass P	mg P kg^{-1}	508	169	S	69	68	NS
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	181	195	NS	32	23	S
CH_4 Production	mg C kg^{-1} d^{-1}	710	454	S	133	216	NS
PMN	mg N kg^{-1} d^{-1}	383	257	S	122	101	NS
PMP	mg P kg^{-1} d^{-1}	144	8	S	10	2	S

NS = not significant at $P<0.05$; S = significant at $P<0.05$

Table 5.4. Various 0-3 cm soil parameters in P-impacted and unimpacted reference areas of Water Conservation Area-1 (WCA-1), and WCA-2a. Means averaged over the various seasonal sampling periods are presented. Significant differences between P-impacted and reference areas of the respective hydrologic units are noted.

Parameter	Units	WCA-1			WCA-2a		
		P-Impacted (n=26)	Reference (n=20)	(P<0.05)	P-Impacted (n=21)	Reference (n=18)	(P<0.05)
Loss on Ignition	%	82	91	S	85	80	NS
Extractable Organic C	g C kg^{-1}	3	6	S	4	3	NS
Extractable NH_4-N	mg N kg^{-1}	317	266	NS	127	116	NS
$NaHCO_3$-P*i*	mg P kg^{-1}	37	5	S	38	7	S
Total P	mg P kg^{-1}	1220	553	S	1140	519	S
Total Inorganic P	mg P kg^{-1}	494	194	S	349	189	S
Total N	g N kg^{-1}	31	36	NS	28	26	NS
Total C	g C kg^{-1}	406	444	S	426	405	NS
Microbial Biomass C	g C kg^{-1}	14	10	NS	6	8	NS
Microbial Biomass N	mg N kg^{-1}	2030	1320	NS	824	776	NS
Microbial Biomass P	mg P kg^{-1}	237	225	NS	146	140	NS
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	39	53	NS	56	43	NS
Aerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	60	26	NS	28	21	NS
Anaerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	27	18	NS	32	18	S
PMN	mg N kg^{-1} d^{-1}	158	122	NS	65	49	NS
PMP	mg P kg^{-1} d^{-1}	24	15	NS	10	6	S

NS = not significant at P<0.05; S = significant at P<0.05

Table 5.5. Various 0-3 cm soil parameters in P-impacted and unimpacted reference areas of Water Conservation Area-3a (WCA-3a) and Taylor Slough. Means averaged over the various seasonal sampling periods are presented. Significant differences between P-impacted and reference areas of the respective hydrologic units are noted.

Parameter	Units	WCA-3a			Taylor Slough		
		P-Impacted (n=33)	Reference (n=30)	($P<0.05$)	P-Impacted (n=60)	Reference (n=12)	($P<0.05$)
Loss on Ignition	%	81	77	NS	30	38	S
Extractable Organic C	g C kg^{-1}	6	5	NS	3	2	S
Extractable NH_4-N	mg N kg^{-1}	168	94	S	68	44	S
$NaHCO_3$-P*i*	mg P kg^{-1}	60	5	S	4	0	S
Total P	mg P kg^{-1}	1280	371	S	306	200	S
Total Inorganic P	mg P kg^{-1}	446	125	S	122	75	S
Total N	g N kg^{-1}	31	34	NS	10	27	S
Total C	g C kg^{-1}	389	412	NS	205	331	S
Microbial Biomass C	g C kg^{-1}	8	5	S	7	5	S
Microbial Biomass N	mg N kg^{-1}	1140	326	S	460	298	S
Microbial Biomass P	mg P kg^{-1}	277	58	S	61	52	NS
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	98	84	NS	43	22	S
CH_4 Production	mg C kg^{-1} d^{-1}	258	165	S	158	57	S
PMN	mg N kg^{-1} d^{-1}	75	27	S	26	10	S
PMP	mg P kg^{-1} d^{-1}	24	4	S	3	1	S

NS = not significant at $P<0.05$; S = significant at $P<0.05$

concentrations in WCA-3 and TS were higher in P-impacted areas. Extractable NH_4-N concentrations were considerably higher in WCA-1 than in other hydrologic units, and were also significantly higher in floc than in underlying 0-3 cm soil for all hydrologic units. Extractable TOC was also significantly related to extractable NH_4-N in both floc and underlying 0-3 cm soil (Tables 5.6-5.7).

The TN content of floc and 0-3 cm soil was generally not affected by P loading or soil depth (Tables 5.2-5.5). However, TS sites exhibited significantly lower TN concentrations than did the other hydrologic units in both floc and 0-3 cm soil. The TC concentrations of floc and soil exhibited mixed response to P loading, though TC concentrations were lower in TS than in other hydrologic units.

The various sites and transects within the four hydrologic units were statistically separated based on floc and soil TP concentrations into P-impacted and non-impacted areas. The impacts of nutrient loading in WCA-1 extended up to 0.5 km from the primary water inflow point. Sites greater than 0.46 km from the inflow that did not exhibit enhanced floc or soil TP concentrations were referred to as P-unimpacted. The extent of nutrient loading in WCA-2a was greater than in other hydrological units, with P-impacted sites being located within 7 km of the S10-C water inflow point. The P-impacted area of WCA-3a consisted of sites within 3 km east or west of the canal, while TP concentrations in TS were lower than in other hydrologic units and exhibited greater variability within the wetland. The TP concentrations in TS were highest within 0.4 km of the water inflow point and significantly decreased several km from the inflow. Interior sites 3-12 km from inflow were variable in TP concentrations and were somewhat higher than some sites within 0.5-3 km of inflow. However, sites 12-24 km from the inflow

Table 5.6. Significant correlation coefficients (P <0.05) in Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough floc (n=342). The PMN and PMP refer to potentially mineralizable N and P, respectively.

Parameter	LOI	Ext. TOC	NH_4-N	$NaHCO_3$-P*i*	TP	TP*i*	TN	TC	MBC	MBN	MBP	PMN	PMP	SOD
Extractable NH_4-N		0.57						0.91		0.62				
$NaHCO_3$-P*i*			0.35											
Total P	0.55			0.66							0.75		0.59	
Total Inorganic P	0.45			0.55	0.94					0.39	0.84	0.43	0.71	
Total N	0.65	0.37			0.51	0.48			0.47	0.57	0.56	0.45		
Total C					0.50	0.42	0.93		0.36	0.41	0.44			
Microbial Biomass C		0.42												
Microbial Biomass N	0.44	0.51							0.64					
Microbial Biomass P	0.46								0.37	0.61		0.64		
Soil Oxygen Demand	0.67	0.53	0.49		0.43		0.59	0.57	0.64	0.58	0.36	0.53		
Aerobic CO_2 Prod.		0.52	0.61					-0.40	0.63	0.66		0.51		0.62
Anaerobic CO_2 Prod.		0.83	0.89		-0.50	-0.47			0.48	0.57		0.81		
CH_4 Production	0.67	0.71	0.69	0.66	0.66	0.58	0.82	0.78		0.63	0.60	0.59	0.41	0.58
PMN	0.37	0.57	0.66						0.66	0.74				
PMP				0.34							0.64	0.51		

Table 5.7. Significant correlation coefficients ($P<0.05$) in Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough 0-3 cm soil (n=342). The PMN and PMP refer to potentially mineralizable N and P, respectively.

Parameter	LOI	Ext. TOC	NH_4-N	$NaHCO_3$-P*i*	TP	TP*i*	TC	MBC	MBN	MBP	PMN	PMP	SOD	Aerobic CO_2
Extractable NH_4-N		0.73						0.64	0.72					
Total P	0.55			0.79					0.49	0.58		0.47		
Total Inorganic P	0.42			0.68	0.89				0.54	0.62		0.46		
Total N	0.91						-0.82							
Total C	0.98		0.35											
Microbial Biomass C		0.43												
Microbial Biomass N		0.51						0.82						
Microbial Biomass P			0.35					0.41	0.61		0.41			
Soil Oxygen Demand	0.58	0.47	0.50	0.56	0.51	0.40					0.53	0.47		
Aerobic CO_2 Prod.		0.35	0.44	0.47			-0.44	0.45	0.41		0.39	0.53		
Anaerobic CO_2 Prod.		0.59	0.66	0.44	0.39		-0.43	0.65	0.54		0.65	0.74	0.93	0.81
CH_4 Production	0.47	0.49	0.71	0.60	0.56	0.60			0.61	0.54	0.81	0.50	0.43	
PMN		0.68	0.87					0.71	0.80					
PMP		0.42	0.48					0.46	0.53	0.76	0.56			

exhibited low TP concentrations and were referred to as the unimpacted area. The decreases of TP in floc and 0-3 cm soil from WCA-1 and WCA-2a with increasing distance from primary water inflow points are shown in Fig. 5.2. Likewise, TP as a function of distance from inflow in WCA-3a and TS is shown in Fig. 5.3.

Background TP concentrations in unimpacted areas exhibited significant differences among the 4 hydrologic units (Tables 5.2-5.5). Floc TP was significantly lowest at the TS unimpacted area while WCA-1 background TP concentrations were higher than at other hydrologic units. In soil, TS had the lowest background TP levels but the other units had similar background TP concentrations in unimpacted areas. In floc, the P-impacted areas of WCA-1 and WCA-3a had significantly higher TP than P-impacted areas of WCA-2a and TS. In soil, the TS P-impacted area had significantly lower TP than other hydrologic units. For all hydrologic units, the TP was significantly higher in floc than in underlying soil for impacted areas. However, in unimpacted areas, the floc TP concentrations were similar to 0-3 cm soil TP concentrations.

The $NaHCO_3$-P*i* was significantly enhanced by P loading in both floc and soil except in TS, where concentrations were approximately 1 mg P kg^{-1} (Tables 5.2-5.5). The $NaHCO_3$-P*i* concentrations were significantly higher in 0-3 cm soil than in floc in WCA-1 and TS impacted areas, but not in other hydrologic units. Background $NaHCO_3$-P*i* concentrations in both floc and 0-3 cm soil were generally <7 mg P kg^{-1} in unimpacted areas of all 4 hydrologic units. However, in P-impacted areas, WCA-3a floc and soil exhibited the highest $NaHCO_3$-P*i* concentrations and TS the lowest ($P<0.05$). The $NaHCO_3$-P*i* contributed approximately less than 1% of the TP in WCA-1 and TS floc and soil. The $NaHCO_3$-P*i* was approximately 3.5% of the TP in WCA-2a floc and soil in P-

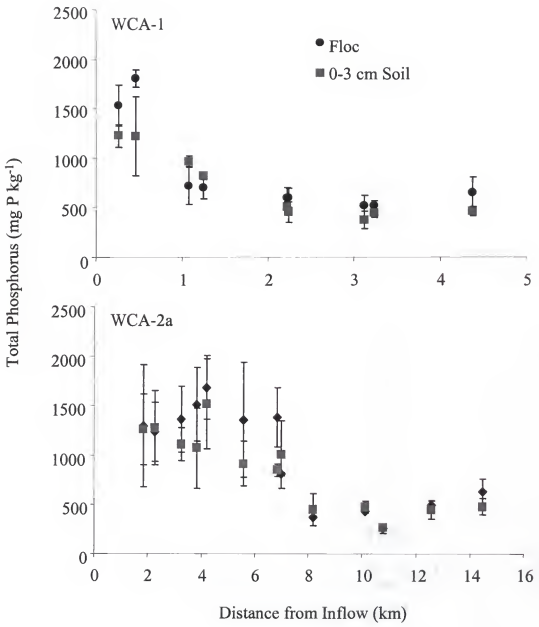

Figure 5.2. Floc and 0-3 cm soil TP concentrations in Water Conservation Area-1 (WCA-1) and WCA-2a as a function of distance from the primary water inflow point.

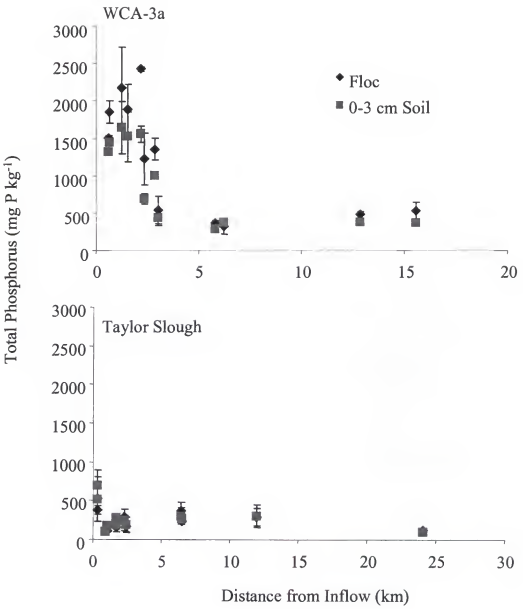

Figure 5.3. Floc and 0-3 cm soil TP concentrations in Water Conservation Area-3a (WCA-3a) and Taylor Slough as a function of distance from the primary water inflow point.

impacted areas, but in unimpacted areas, $NaHCO_3$-P*i* was <1% of TP. In WCA-3a, $NaHCO_3$-P*i* was 4-8% of TP in P-impacted areas but only about 1% in unimpacted areas.

The TP*i* represented the acid-soluble inorganic P fraction in soil. The TP*i* was significantly enhanced by P loading for all 4 hydrologic units in both floc and underlying soil (Tables 5.2-5.5). Floc TP*i* was approximately 2-3 times greater in P-impacted areas than in unimpacted areas for all 4 hydrologic units. The TP*i* was significantly lowest in TS impacted and unimpacted areas in both floc and soil. The TP*i* contributed approximately 30-45% of the TP in both floc and 0-3 cm soil of all 4 hydrologic units. The percentage of TP*i* in TP was not influenced by P loading (distance from the primary water inflow point) or by soil depth.

Microbial Biomass

In floc, MBC was significantly higher in unimpacted than P-impacted areas of WCA-1 and WCA-3a, while no differences were observed in WCA-2a and TS floc (Tables 5.2-5.5). In soil, MBC was generally higher in P-impacted areas than in unimpacted areas. In floc, MBC was significantly highest in WCA-3a and lowest in TS. In soil, there were no differences in MBC among the hydrologic units, however, MBC in floc was over twice as high as underlying 0-3 cm soil.

The MBN of floc was not significantly enhanced by P loading (Tables 5.2-5.5). However, in soil, MBN was significantly higher in impacted areas of WCA-3a and TS than in unimpacted areas. Floc MBN was over twice as high as soil MBN in the 4 hydrologic units. In addition, TS had significantly lower MBN than other hydrologic units.

The MBP of floc was significantly enhanced by P loading in WCA-2a and WCA-3a (Tables 5.2-5.5). In underlying soil, the MBP was significantly enhanced by P loading, but only in WCA-3a. The MBP was almost twice as high in floc as in underlying 0-3 cm soil. Taylor Slough sites exhibited the lowest MBP among the various hydrologic units while WCA-1 exhibited the highest background MBP for both floc and 0-3 cm soil.

Microbial Processes

The SOD was generally not affected by P loading in either floc or 0-3 cm soil, except in WCA-1 floc and TS floc and soil (Tables 5.2-5.5). However, SOD was significantly higher in floc than in 0-3 cm soil for all hydrologic units. The WCA-3a had the highest SOD in floc and soil among the hydrologic units ($P<0.05$). Aerobic and anaerobic CO_2 production rates were generally not impacted by P loading in either floc or soil, although variability among sites was high (Tables 5.2-5.5). The CO_2-production rates were not influenced by soil depth, but were significantly higher in WCA-1 than WCA-2a. Methane-production rates were significantly higher in P-impacted areas than unimpacted areas in WCA-3a floc and soil and TS soil (Tables 5.2-5.5). In WCA-3a, CH_4-production rates were higher in floc than in 0-3 cm soil, but no differences between floc and soil CH_4 production rates were observed in TS.

The PMN rates in WCA-3a and TS floc and 0-3 cm soil were significantly higher in P-impacted than in unimpacted areas (Tables 5.2-5.5). However, no differences in PMN rates between P-impacted and reference areas were observed for WCA-1 and WCA-2a. Taylor Slough exhibited the lowest PMN rates among the various hydrologic

units, and PMN was also significantly higher in floc than in underlying soil for all of the hydrologic units.

The PMP was significantly enhanced by P loading in both floc and 0-3 cm soil (Tables 5.2-5.5). Background PMP levels in P-unimpacted areas ranged from up to 15 mg P kg^{-1} d^{-1} in floc and soil for all hydrologic units. The PMP was also significantly higher in floc of P-impacted areas than in soil from P-impacted areas. The WCA-3a exhibited the highest PMP in floc, while TS exhibited the lowest PMP in both floc and soil.

Relationships between Biogeochemical Indicators and Microbial Processes

The microbial biomass C, N, and P were often significantly related to one another in floc and soil (Tables 5.6 and 5.7). The microbial biomass was also significantly related to various chemical parameters such as extractable nutrient concentrations. The MBC was significantly related to extractable TOC concentrations in both floc and 0-3 cm soil. In addition, the MBN was related to both extractable TOC and NH_4-N in floc and 0-3 cm soil. The MBP was significantly correlated with both TP and TP*i* in floc and soil (Tables 5.6 and 5.7). Indeed, most of the variability in microbial biomass in floc and soil was explained by extractable TOC, NH_4-N, or various inorganic P fractions (Table 5.8).

Various heterotrophic microbial activities such as SOD, CH_4-, and CO_2-production rates were significantly related to various parameters including extractable TOC and microbial biomass C and N in floc and soil. The SOD was significantly correlated with various parameters such as extractable TOC and MBC (Tables 5.6 and 5.7), and the best predictors of SOD in floc and soil were MBC and TP (Table 5.8). Methane production rates were significantly correlated with many biogeochemical

Table 5.8. Significant regression equations ($P<0.05$) for various biogeochemical indicators in floc and soil from Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough. The MBC = microbial biomass C; MBN = microbial biomass N; MBP = microbial biomass P; PMN = potentially mineralizable N; PMP = potentially mineralizable P; SOD = soil oxygen demand; CH_4 = methane production rate; CO_2 = carbon dioxide production rate; TP*i* = total inorganic P; TP = total P; LOI = loss on ignition, TOC = total organic carbon.

Regression Equation	R^2
Floc (n = 342)	
MBC = 4520 + 1.2 (ext. TOC) - 25 (NH_4-N) + 9.1 (MBN)	0.45
MBN = 108 + 0.77 (ext. TOC) + 3.2 (NH_4-N) + 4.4 (MBP)	0.59
MBP = -39 + 0.03 (MBN) - 0.7 ($NaHCO_3$-P*i*) + 0.8 (TP*i*)	0.81
SOD = 7.7 + 0.2 (NH_4-N) + 0.001 (MBC) + 0.03 (TP)	0.63
Aerobic CO_2 = 16 + 0.001 (ext. TOC) + 0.0004 (MBC)	0.49
Anaerobic CO_2 = -0.9 + 0.002 (ext. TOC) + 0.1 (NH_4-N)	0.82
CH_4 = -30 + 0.0007 (MBC) + 0.4 (MBP) + 5.8 (TN)	0.93
PMN = 39 + 0.3 (NH_4-N) + 0.002 (MBC)	0.66
PMP = -32 - 0.1 ($NaHCO_3$-P*i*) + 0.2 (TP*i*)	0.51
0-3 cm Soil (n = 342)	
MBC = 4000 - 3.6 (TP) + 7.5 (MBN)	0.71
MBN = -305 + 0.1 (MBC) + 0.6 (TP)	0.77
MBP = -24 + 0.1 (MBN) + 0.1 (TP)	0.47
SOD = -0.3 - 0.4 (TN) + 0.01 (ext. C) + 0.001 (MBC) + 0.02 (TP)	0.90
Aerobic CO_2 = 91 + 3.5 (LOI) + 0.4 ($NaHCO_3$-P*i*) - 0.9 (TC)	0.72
Anaerobic CO_2 = 1.2 + 0.05 (NH_4-N) + 0.5 ($NaHCO_3$-P*i*) + 0.003 (MBC) - 0.02 (MBN)	0.72
CH_4 = 25 + 0.02 (ext. TOC) + 0.1 (TP)	0.71
PMN = -24 + 0.4 (NH_4-N) + 0.04 (MBN)	0.83
PMP = -4.3 + 0.03 (NH_4-N) + 0.08 (MBP)	0.63

indicators, including extractable TOC and microbial biomass (Tables 5.6 and 5.7). Extractable TOC and NH_4-N, in addition to microbial biomass, were significantly related to CO_2-production rates in floc and soil (Tables 5.6 and 5.7). In floc, extractable TOC was a good indicator of CO_2-production rates (Table 5.8).

The PMN was significantly correlated with MBC and extractable NH_4-N (Tables 5.6 and 5.7). Extractable NH_4-N and microbial biomass explained most of the variability in PMN rates in both floc and soil (Table 5.8), and the PMP was significantly correlated with TP and TP*i* in floc and soil (Tables 5.6 and 5.7). Most of the variability in PMP was explained by various P fractions and by MBP (Table 5.8).

Impact Index

A relative P impact index was developed to allow for biogeochemical indicators to be expressed on a unitless basis. This simplifies comparison of P impacts (Table 5.9). The formula for selected parameters is:

$$\text{Impact Index} = \log [(\text{P-impacted area}) / (\text{reference area})].$$

The most consistent indicators in floc and 0-3 cm soil which showed enhancement due to P loading were P-related parameters such as TP, TP*i*, MBP, and PMP. In a small number of cases, certain indicators proved higher in unimpacted areas than P-impacted areas, and were designated with a negative number.

Discussion

All four hydrologic units exhibited increases in floc and soil TP due to nutrient loading. However, the extent of the impacted areas within the respective hydrologic units was variable. Based on TP concentrations, impacts in WCA-1 were located within 0.5 km of water inflow points around the periphery of the wetland. However, in WCA-2a,

Table 5.9. Impact index [log (P-impacted area/reference area)] for various biogeochemical indicators in floc and soil for Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough. The PMN and PMP refer to potentially mineralizable N and P, respectively.

Parameter	WCA-1	WCA-2a	WCA-3a	TS
Floc				
Loss on Ignition	0.0	0.0	0.1	0.1
Extractable Organic C	-0.3	-0.2	0.0	0.0
Extractable NH_4-N	0.0	-0.2	0.1	0.0
$NaHCO_3$-*Pi*	0.4	1.3	1.5	0.1
Total P	0.4	0.5	0.6	0.3
Total Inorganic P	0.5	0.5	0.6	0.3
Total N	0.0	0.0	0.0	-0.1
Total C	0.0	0.0	0.0	-0.1
Microbial Biomass C	-0.3	-0.2	-0.2	-0.1
Microbial Biomass N	-0.1	-0.2	0.1	0.2
Microbial Biomass P	0.2	0.3	0.5	0.3
Soil Oxygen Demand	0.2	-0.1	0.0	0.2
Aerobic CO_2 Production	0.0	-0.1	-	-
Anaerobic CO_2 Production	-0.4	0.0	-	-
CH_4 Production	-	-	0.2	-0.2
PMN	0.0	-0.1	0.2	0.2
PMP	0.6	0.4	1.2	1.0
0-3 cm Soil				
Loss on Ignition	0.0	0.0	0.0	0.2
Extractable Organic C	-0.3	0.1	0.1	0.3
Extractable NH_4-N	0.1	0.0	0.3	0.2
$NaHCO_3$-*Pi*	0.9	0.7	1.1	1.0
Total P	0.3	0.3	0.5	0.4
Total Inorganic P	0.4	0.3	0.6	0.4
Total N	-0.1	0.0	0.0	0.2
Total C	0.0	0.0	0.0	0.1
Microbial Biomass C	0.1	-0.1	0.2	0.2
Microbial Biomass N	0.2	0.0	0.5	0.4
Microbial Biomass P	0.0	0.0	0.7	0.5
Soil Oxygen Demand	-0.1	0.1	0.1	0.3
Aerobic CO_2 Production	0.4	0.1	-	-
Anaerobic CO_2 Production	0.2	0.2	-	-
CH_4 Production	-	-	0.2	0.4
PMN	0.1	0.1	0.4	0.5
PMP	0.2	0.2	0.7	0.4

the extent of P loading covered a much larger geographic scale, as the P-impacted area extended up to 7 km from the inflow. The extent of P movement may be due both to water movement through the hydrologic units and to mass P loading (McCormick et al, 2000; Newman et al., 2001). The limited P-impacted area in WCA-1 may be due to lack of water flow from the periphery to the interior of the wetland. Indeed, the hydrology of WCA-1 is primarily driven by rainfall (McCormick et al., 2000). Water movement in WCA-2a is generally in a north to south direction from P-impacted areas adjacent to water inflow points to unimpacted interior areas (McCormick et al., 2000). The P-impacted areas of the southernmost hydrologic units, WCA-3a and TS, also generally exhibited smaller impact zones than WCA-2a.

Assessment of the P-impacted area in TS was difficult due to the low TP levels observed in addition to variability between replicate sampling. An area within 0.5 km of the inflow exhibited high TP levels, followed by a decrease up to 3 km. The TP levels increased again in the interior of TS at a distance of 3-12 km from the inflow. However, sites greater than 12 km from the inflow exhibited the lowest TP concentrations. Perhaps these areas exhibiting lower TP levels which were located only 0.5 km to 3 km from inflow were hydrologically isolated, thus restricting movement of P-laden water, and subsequently limiting their exposure to P.

The WCA-1 and WCA-2a areas were sampled 3 times over a 17-month period (data not shown), and significant increases in floc TP were observed at P-impacted sites in WCA-1 over this period. In addition, TP concentrations in WCA-2a significantly increased at sites 5.6 – 7.0 km from inflow during the 17-month sampling period (data not shown). Increased TP levels may be due to continued P loading into WCA-1 and

WCA-2a from the EAA. This may explain increases in soil TP in the periphery of WCA-1 but not for WCA-2a, because TP increases over the sampling period were not observed in the impacted areas nearest the water inflow point of WCA-2a. It is possible that P regeneration from organic matter degradation from sites located 5.6 km from inflow in WCA-2a was responsible for increased TP levels observed from 5.6-7 km south of the water inflow point. Indeed, nutrient regeneration from organic soils may be as important as external nutrient loading (Verhoeven et al., 1988). An alternative explanation may be that enhanced organic matter accumulation, primarily recently deposited *Typha* plant tissue, in P-impacted areas located within several km of the S-10C water inflow point of WCA-2a, has retarded water movement through these sections of WCA-2a, thus channelizing water around the highly P-impacted areas into areas with less organic matter accumulation, including sites located within 5.6 km from the water inflow point. In addition, the accumulation of detrital organic matter in P-impacted areas may result in exposure of surface detritus to aerobic decomposition during periods of drought, thus enhancing organic matter degradation and nutrient regeneration and increasing the supply of nutrients to previously unimpacted areas.

The most sensitive indicators of P loading in floc and 0-3 cm soil were P-related soil biogeochemical indicators such as TP, TP*i*, $NaHCO_3$-P*i*, and PMP. However, $NaHCO_3$-P*i* concentrations were often very low and somewhat variable over time, so $NaHCO_3$-P*i* may not be an adequate indicator of P enrichment. Other indicators of P enrichment, such as heterotrophic microbial activities, were often enhanced in P-impacted areas (Newman et al., 2001; Wright and Reddy, 2001a,b). However, these processes also were often regulated by other factors such as substrate quality and

availability of electron acceptors and nutrients (Fenchel and Jorgensen, 1977; Amador and Jones, 1993; D'Angelo and Reddy, 1999; Wright and Reddy, 2001a,b).

The northern most hydrologic units have been more directly impacted by P loading from the EAA for a longer duration than the southernmost hydrologic units, such as TS (McCormick et al., 2000). Microbial biomass C, N, and P were often significantly enhanced by P loading, especially in TS floc and soil. Taylor Slough had the lowest background TP levels of the 4 hydrologic units and thus had limited exposure to P loading. Therefore, P loading into the initially low TP soils of TS provoked a significant response in microbial biomass C, N, and P in floc and in underlying soil. Thus, potential impacts of nutrient loading on various biogeochemical indicators appears dependent at least in part on background floc and soil TP levels.

Phosphorus loading enhanced various P fractions in floc and soil, such as TP, TP*i*, MBP, and PMP. These biogeochemical indicators were more sensitive and directly affected by P loading. Other processes such as microbial biomass were not only related to P loading but also various soil chemical parameters including extractable nutrient concentrations. Furthermore, various heterotrophic microbial activities such as SOD, CH_4, and CO_2-production rates, appeared to be controlled by microbial biomass in addition to P loading and extractable nutrient concentrations. The microbial biomass was often correlated and predicted by various soil chemical parameters such as extractable TOC for MBC, extractable NH_4-N for MBN, and TP and TP*i* for MBP. In addition, various heterotrophic microbial activities including SOD, CH_4, and CO_2-production rates were strongly correlated with and predicted by MBC and extractable TOC.

Extractable NH_4-N concentrations were almost 5 times higher in WCA-1 than in other hydrologic units. A likely cause is that WCA-1 received drainage water from the EAA whereas other hydrologic units, particularly WCA-3a and TS, received water input from the northern WCAs. Nutrient loading from the EAA and restricted water movement into the interior of WCA-1 may have been responsible for the increased NH_4-N concentrations in the peripheral P-impacted areas (McCormick et al., 2000). In addition, heterotrophic microbial activity as measured by CO_2 production and PMN was generally higher in WCA-1 than in WCA-2a, suggesting that enhanced organic matter degradation in WCA-1 resulted in increased NH_4-N accumulation in soil. Similar increases in soil NH_4 availability were observed in P-impacted soil (White and Reddy, 2000; Newman et al., 2001). Thus, P loading has a marked impact on N cycling and on nutrient regeneration in P-impacted soils. However, northern hydrologic units such as WCA-1, were exposed not only to P loading but also to N loading in both inorganic and organic forms (McCormick et al., 2000). Both N and P loading may contribute to changes in soil biogeochemical indicators and to increased NH_4-N concentrations in WCA-1.

Surface floc was more sensitive to P loading than was the underlying 0-3 cm soil. Most biogeochemical indicators, including microbial biomass and related heterotrophic microbial activities, were highest in the surface floc and decreased with depth. Similar decreases in microbial activities with depth have been reported in Everglades soils (White and Reddy, 2000; Newman et al., 2001; Wright and Reddy, 2001a,b). The floc consisted of benthic periphyton, while the soil consisted primarily of consolidated and decomposed plant matter. Thus, the enhanced substrate quality of the floc likely contributed to enhanced microbial biomass and heterotrophic microbial activities compared to the

underlying soil (DeBusk and Reddy, 1998). In addition, the floc sediments were often exposed to O_2 during periods of drought and low water input, thus supporting aerobic microbial processes and enhanced heterotrophic microbial activities. The response of various biogeochemical indicators to nutrient loading also varied considerably with soil depth.

Conclusions

Phosphorus loading has led to the development of distinct gradients in floc and soil TP in several hydrologic units of the Everglades from areas near water inflow points to the interior of the wetlands. However, the extent of P impacts in the hydrologic units was variable and dependent on mass P loading and hydrologic conditions.

The most sensitive biogeochemical indicators of P loading were various P-related parameters such as TP, TP*i*, $NaHCO_3$-P*i*, and PMP. Various other indicators were enhanced by P loading but appeared also to be controlled in part by soil chemical parameters including extractable TOC and NH_4-N, in addition to microbial biomass. Background TP concentrations were important for estimating potential P impacts on microbial processes, with wetlands having low background TP levels being more susceptible to changes in microbial biomass and heterotrophic microbial activities. Seasonal sampling over a 17-month period showed that TP concentrations were increasing in P-impacted areas, particularly in WCA-1 and WCA-2a, which may be due both to external nutrient loading and internal P regeneration from organic matter degradation. Additional studies in the southernmost hydrologic units, WCA-3a and TS, should provide a more detailed assessment of soil TP concentrations and of the extent of P loading and subsequent impacts on biogeochemical indicators.

CHAPTER 6
BIOGEOCHEMICAL INDICATOR RESPONSES TO PHOSPHORUS ENRICHMENT IN SELECTED HYDROLOGIC UNITS OF THE EVERGLADES

Introduction

Impacts of nutrient loading or enrichment on vegetation and microbial community structure and dynamics have been documented in wetland and aquatic ecosystems. The addition of limited nutrients to ecosystems often results in enhanced productivity of vegetation and stimulation of the microbial community (Chrost, 1991; Craft and Richardson, 1995; Chiang et al., 2000; Newman et al., 2001). However, the microbial community may be more sensitive or respond more readily to increases in nutrient levels than do vegetational communities. Changes in vegetation patterns due to nutrient loading may take years to be observed (Chiang et al., 2000; McCormick et al., 2000), while microbial communities and associated processes may be altered after only a short exposure to increased nutrient levels.

Nutrient loading into oligotrophic systems often results in enhanced microbial biomass, which in turn is responsible for enhanced organic matter degradation and nutrient cycling (White and Reddy 2000; Wright and Reddy, 2001a,b). An understanding of the impacts of nutrient loading on microbial processes is important, because organic matter degradation and nutrient cycling are dependent on the chemical and physical composition of organic matter, microbial community composition, and availability of nutrients (Webster and Benfield, 1986; Rybczyk et al., 1996). Microbial biomass is

considered the key component regulating organic matter degradation and nutrient cycling (Wardle, 1992; Martens, 1995).

Historically, the Florida Everglades wetland ecosystems developed as relatively nutrient-poor systems and supported vegetation adapted to these conditions (Davis, 1943). Over the past century, the Everglades has been drained and divided by levees and canals and separated into various distinct hydrologic units where water movement and storage is strictly regulated. These units include the Everglades Agricultural Area (EAA), Water Conservation Area-1 (WCA-1), WCA-2a, and WCA-3a, and Taylor Slough (TS) of the Everglades National Park (ENP) (Maltby and Dugan, 1994). External nutrient loading has been implicated in altering the Everglades wetland ecosystems by increasing soil nutrient levels (particularly P), which has promoted expansion of *Typha*, particularly in WCA-2a (Davis, 1991; Koch and Reddy, 1992; DeBusk et al., 1994). Most impacts associated with P enrichment have been observed in areas adjacent to surface water inflow points or canals. The impacts of external nutrient loading to the Everglades is documented in the distribution of floodwater and soil total phosphorus (TP) (DeBusk et al., 1994; Newman et al., 1997; Reddy et al., 1998).

In addition to contributing to changes in vegetation patterns in the Everglades (Craft and Richardson, 1995; Richardson et al., 1997; Miao and Sklar, 1998), P loading has altered various soil chemical and microbial processes, both stimulatory and negative to some degree (Amador and Jones, 1993; McCormick and O'Dell, 1996; Newman et al., 1997; Reddy et al., 1998; Wright and Reddy, 2001a,b). Microbial biomass, heterotrophic microbial activity, and various enzyme activities have been enhanced or depressed at P-impacted sites in various hydrologic units (Amador and Jones, 1993; DeBusk and Reddy,

1998; White and Reddy, 1999; Wright and Reddy, 2001a,b). However, these changes in microbial community dynamics may also be regulated in part by other variables such as vegetation or hydrologic conditions (McCormick et al., 2000). In this study we report the results of experiment enrichment of oligotrophic wetlands with P loading to elucidate the effects on soil biogeochemical properties and on soil microbial communities.

The objectives of the this study were to: 1) determine the impacts of P dosing on soil biogeochemical parameters; 2) identify sensitive soil biogeochemical indicators of P enrichment as a function of both soil depth and P loading rates; and 3) determine the relationships between biogeochemical indicators and microbial activities.

Materials and Methods

Site Description

Experimental mesocosms were established in oligotrophic slough areas of four hydrologic units of the Everglades (WCA-1, WCA-2a, WCA-3a, and TS) to determine the effects of P dosing on soil biogeochemical indicators.

The WCA-1 is a national wildlife refuge encompassing 59,000 ha of the northern Everglades. Rainfall is its primary water input, while remaining sources of water include P-laden runoff from the EAA. Most of the increased soil TP levels have been observed in areas adjacent to canals or levees (Newman et al., 1997). *Typha* predominates in P-impacted areas while *Cladium*, open sloughs, and tree islands are common in P-unimpacted areas.

The WCA-2a (44,700 ha) receives drainage water from WCA-1 in addition to discharge water from the EAA. The impact zone in WCA-2a is much broader and extends much farther into its interior than the impact zone in WCA-1, thus P impacts on

vegetation community structure are more evident in WCA-2a (DeBusk et al., 2001a). The WCA-2a vegetation consists of *Typha* in P-impacted areas, while *Cladium* and sloughs dominate in the P-unimpacted interior areas.

The WCA-3a receives drainage water from northern sites, particularly WCA-2a, though rainfall contributes approximately 42% of its annual water budget (Reddy et al., 1998). In WCA-3a, tree islands and wet prairies comprise the primary vegetation community structure, while most areas near water-inflow structures exhibit enhanced soil TP concentrations.

Taylor Slough is located south of WCA-3a and serves as a bridge with Florida Bay, and TS receives discharge water from WCA-3a. The soils of TS differs from that of other hydrologic units, as the wet prairies in TS formed on marl sediments under P-limited conditions.

Soil Sampling

The South Florida Water Management District (SFWMD) established several experimental mesocosms in oligotrophic sloughs located in P-unimpacted interior areas of WCA-1, WCA-2a, WCA-3a, and TS. These consisted of plastic circular tanks (2.5 m^2) with each being replicated 3 times. In addition, open controls were also included to determine the mesocosm enclosure effect. Mesocosms were spiked weekly with various amounts of NaH_2PO_4 mixed with site water to achieve desired loading rates. The tanks were closed for 24 hr after spiking to allow for P in the floodwater to be taken up by benthic periphyton and other biota. The mesocosms were then opened to permit exchange with the surrounding water for the remainder of the week, until the next weekly P dosing. A total of 21 mesocosms were established in WCA-1 and dosed starting in

March 1996 at rates of 0, 0.2, 0.4, 0.8, 1.6, and 3.2 g P m^{-2} yr^{-1}. Floc and underlying 0-3 cm soil were then sampled during May 1997, March 1998, and October 1998. Phosphorus dosing to WCA-2a mesocosms (total of 24) was initiated during June 1995 at rates of 0, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 g P m^{-2} yr^{-1}. Floc and 0-3 cm soil were then sampled during April 1997, March 1998, and September 1998.

In WCA-3a and TS, duplicate interior locations were used to establish mesocosms. Sites were chosen in the northern and southern sections of these hydrological units and were designated as WCA-3a-north and WCA-3a-south and TS-north and TS-south. The mesocosm sites were initially dosed beginning in November 1999 at rates of 0, 0.2, 0.8, and 3.2 g P m^{-2} yr^{-1}. The WCA-3a mesocosms were sampled in February and October 2000, while TS mesocosms were sampled in March and October 2000.

Floc and soil samples in mesocosms were collected by driving a 5.1 cm diameter plastic corer into soil to a depth of 10 cm. The top 3 cm of each core were sectioned for analysis. A soil depth of 0-3 cm was selected to minimize the dilution of added P. Floc samples were taken from above the cored soil and all samples were stored at 4°C until analysis.

Methods of Analysis

Physico-Chemical Properties

In the laboratory, samples were weighed, homogenized thoroughly, and 20 g subsamples were dried at 70°C for 72 hr to determine the percent solids. Soil bulk density was then calculated for soil on a dry-weight basis. Total carbon (C) and nitrogen (N) content of floc and soil were determined on dried, ground samples using a Carlo-Erba

NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Loss on ignition (LOI) was determined as the mass loss of soil after ashing at 550°C divided by the initial sample mass.

Extractable ammonium (NH_4-N) was determined by shaking wet soil samples with 20 ml of 0.5 *M* K_2SO_4 for 1 hr on a longitudinal shaker. Samples were then centrifuged for 10 min at 6000 rpm, and vacuum-filtered through Whatman #42 filter paper. The supernatant was collected and NH_4-N was determined colorimetrically (U. S. EPA, 1993b).

Soil TP was determined using an ashing method (Anderson, 1976). Approximately 0.2 g oven-dried soil was placed in a muffle furnace for 3-4 hr at 550°C. Samples were then acidified using 20 mL of 6 *M* HCl and digested for several hours until dry. Samples were then re-dissolved in 2.25 mL of 6 *M* HCl, filtered through Whatman #41 filter paper, and brought to a final volume of 50 ml. The TP was then determined using an automated colorimetric procedure (U. S. EPA, 1993c). Soil total inorganic P (TP*i*) was determined by extracting 0.5 g dried, ground soil with 25 mL of 1.0 *M* HCL for 3 hr, followed by vacuum filtration using 0.45 µM membrane filters (Reddy et al., 1998) and colorimetric analysis (U. S. EPA, 1993c).

Microbial Biomass

Microbial biomass C (MBC) was determined by the fumigation-extraction method of Vance et al. (1987). Approximately 5 g samples were placed into polypropylene centrifuge tubes, 0.5 mL volume of chloroform was added, and tubes were placed into a vacuum desiccater with a beaker containing additional chloroform. Air in the desiccater was evacuated three times until the chloroform began boiling. Each time,

air was allowed back into the desiccater by means of a screw control valve on its lid. After the third evacuation, the desiccater was sealed under a vacuum for 24 hr. An non-fumigated control sample set was placed on the adjacent lab bench. After 24 hr, samples were removed and both the controls (not exposed to chloroform) and chloroform treated samples were extracted with 20 ml of 0.5 *M* K_2SO_4, shaken for 1 hr on a longitudinal shaker, and vacuum-filtered through #42 Whatman filter paper. Samples were then analyzed for total organic carbon (TOC) using a Dohrmann TOC analyzer (Rosemount Analytical, Santa Clara, CA). Microbial biomass carbon was determined by subtracting TOC of the controls from the TOC of chloroform-treated samples. An extraction efficiency (k_{EC}) factor of 0.37 was applied, utilizing a previous calibration for organic soils by Sparling et al., (1990). Extractable TOC was defined as the TOC from extracted, non-fumigated controls.

Microbial biomass N (MBN) was determined by a fumigation-extraction technique of Brookes et al. (1985). Ten ml of non-fumigated and fumigated extracts from the MBC procedure were subjected to Kjeldahl-N digestion at 380°C for 4-5 hr using the salicylic acid modification of Bremner and Mulvaney (1982). Samples were brought to a final volume of 20 mL after digestion, and transferred into scintillation vials. Extracts were analyzed for total Kjeldahl nitrogen (TKN) colorimetrically (U. S. EPA, 1993b). Microbial biomass N was determined as the difference in TKN between fumigated and non-fumigated samples. A combined extraction efficiency and K*n* value of 0.54 was applied to values when calculating MBN (Brookes et al., 1985).

Microbial biomass P (MBP) was determined as the difference between the TP of 0.5 *M* $NaHCO_3$ extracts of chloroform-fumigated and non-fumigated samples (Ivanoff et

al., 1998). The MBP samples were incubated and fumigated as described for MBC. After a 24 hr incubation, both fumigated and non-fumigated samples were extracted with 20 mL of 0.5 *M* $NaHCO_3$ by shaking for 16 hr. After shaking, samples were centrifuged at 6000 rpm for 10 min, and then filtered through 0.45 μM membrane filters. Portions of the non-fumigated 0.5 *M* $NaHCO_3$ extracts were analyzed for P using an automated colorimeteric method (U. S. EPA, 1993c) and were referred to as $NaHCO_3$-P*i*. Other portions of both fumigated and non-fumigated 0.5 *M* $NaHCO_3$ extracts were digested on a block digester for 4-5 hr at 380°C. These samples were then analyzed colorimetrically (U. S. EPA, 1993c). The difference in TP between fumigated and non-fumigated digested samples was referred to as MBP. No extraction efficiency factor was employed for the calculation of MBP.

Microbial Processes

Soil oxygen demand (SOD) was determined by measuring dissolved oxygen (O_2) depletion during a 24 hr incubation at 20°C. Approximately 2–5 g samples were added to 60 mL bottles followed by addition of O_2-saturated water to volume. The initial dissolved O_2 (DO) content of the slurry was then measured using a YSI Model 58 O_2 meter with a DO probe (Yellow Springs, CO). After 24 hr incubation, samples were stirred and DO content was measured. Soil oxygen demand was calculated as the difference in DO of the O_2 saturated soil:water slurry at t = 0 minus the measured DO at 24 hr, multiplied by water volume and divided by the sample mass.

Aerobic carbon dioxide (CO_2) production rates were determined on samples collected from WCA-1 and WCA-2a mesocosms by measuring CO_2 production over a 10 d incubation period at 30°C (Wright and Reddy, 2001b). Approximately 5-10 g wet

samples were placed into glass bottles with smaller vials containing 3 mL of a 0.5 *M* NaOH trap. Bottles were filled with N_2 for anaerobic incubations. Vials containing the NaOH traps were removed and capped at 2 d intervals up to 10 d. Later, 0.5 mL of 3 *M* HCl was added to the collected vials and CO_2 in the headspace was measured by gas chromatography (Shimadzu GC-8A, thermal conductivity detector at 25°C, Poropak N column at 20°C). Gas pressures in the vials were measured before gas sampling using a digital pressure meter (Kane-May, Great Britain) and subsequently used in the calculation of CO_2 production. Appropriate controls containing no soil were included to account for background CO_2 concentrations.

Methane (CH_4) production rates were determined using 5-10 g wet samples placed into glass serum bottles sealed with butyl rubber septa and incubated at 30°C for 10 d. At time intervals of approximately 2 d, aliquots of headspace were sampled and run on a Shimadzu gas chromatograph-8A GC fitted with a flame ionization detector (160°C), and a Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 110°C. Gas pressure in the vials was measured before gas sampling using a digital pressure meter (Kane-May, Great Britain). Appropriate controls containing no soil were included to account for background CH_4 concentrations.

Potentially mineralizable N (PMN) was determined using anaerobic incubations over a 10 d incubation period (White and Reddy, 2000). Glass serum bottles were prepared by adding 10 g of wet samples and 5 mL of distilled, de-ionized water. Bottles were capped with butyl rubber septa and sealed with aluminum crimps. The headspace was then evacuated and replaced with N_2 gas. Serum bottles were incubated in the dark at 40°C for 10 d. A set of samples were also prepared similarly but without incubation

and served as time 0 controls. Samples were removed from the incubator at the end of incubation and extracted with 20 ml of 0.5 *M* K_2SO_4. Bottles were shaken for 1 hr on a longitudinal shaker, and centrifuged for 10 min at 6000 rpm. The supernatant was filtered through Whatman #42 filter paper, collected in scintillation vials, and analyzed for NH_4-N (U. S. EPA, 1993b). The difference in NH_4-N concentrations between samples incubated for 10 d and concentrations for time 0 controls was referred to as PMN.

Potentially mineralizable P (PMP) was determined using anaerobic incubations over a 10 d period (Chua, 2000). Incubations for PMP were carried out as described for the PMN determinations. The PMP samples were extracted with 20 mL of 1.0 *M* HCl, shaken for 3 hr, centrifuged for 10 min at 6000 rpm, and filtered through 0.45 μM membrane filters, and extracted P was quantified colorimetrically (U. S. EPA, 1993c). The PMP was determined as the difference between P concentrations from the 10 d incubation samples and concentrations from the time 0 controls.

Data Analysis

Data were statistically analyzed using an analysis of variance (ANOVA) model to determine significant differences ($P<0.05$) between P loading rates, sampling time after the onset of P loading, soil depth, and hydrological units. Treatments comparisons were made using Fisher's LSD at $P<0.05$ using CoStat (Minneapolis, MN). Relationships between various chemical and microbial parameters were investigated using linear correlation coefficients (r) at $P<0.05$ to determine the most appropriate indicators of P loading. Mean values for various sampling periods were combined for presentation in tables and figures.

Results

Physico-Chemical Properties

Background concentrations of various parameters in control mesocosms not receiving P dosing are presented in Table 6.1. Statistically significant differences between the highest P-loading rates at the final sampling period and the control mesocosms are presented in Table 6.2. Data among duplicate mesocosm sites in WCA-3a and TS were statistically similar, so data from each were combined for statistical analysis and presentation in tables.

Bulk density of the 0-3 cm soil layer generally ranged from 0.05–0.10 g cm^{-3} for WCA-1, WCA-2a, and WCA-3a and from 0.18-0.26 g cm^{-3} for TS, and was not affected by P loading. Water content of floc sediments was typically >95% for all hydrologic units. Soil water content generally ranged from 90-99% for WCA-1, WCA-2a, and WCA-3a mesocosms and 75-80% for TS mesocosms.

Total C and N contents of floc and soil were not affected by P loading (Table 6.2). However, both TC and TN were higher for WCA-1 and WCA-3a than for WCA-2a and TS. Phosphorus loading had no influence on LOI in floc or soil in WCA-1 or WCA-2a (Table 6.2). The LOI in WCA-1 was approximately 83-91% for floc and soil, while in WCA-2a, LOI averaged 50% for floc and 72% for underlying soil. The LOI of WCA-3a floc and soil was approximately 90% compared to only 39% for TS floc and 20% for TS soil. The calcareous nature of the TS and WCA-2a floc and soil were likely responsible for the lower LOI than observed in WCA-1 and WCA-3a. Extractable TOC in floc and soil was generally not affected by P loading, but was significantly higher in floc than in underlying 0-3 cm soil (Tables 6.1 and 6.2). However, in TS, extractable TOC was

Table 6.1. Background biogeochemical indicator levels (standard error) of various hydrologic units. The PMN and PMP refer to potentially mineralizable N and P, respectively. For Water Conservation Area-1 (WCA-1) and WCA-2a, n=9. For WCA-3a and Taylor Slough, n=12).

Parameter	Units	WCA-1	WCA-2a	WCA-3a	Taylor Slough
Floc					
Loss on Ignition	%	83 (3)	50 (3)	91 (0)	39 (3)
Extractable Organic C	g C kg^{-1}	7 (1)	5 (0)	12 (1)	7 (1)
Extractable NH_4-N	mg N kg^{-1}	-	181 (8)	379 (40)	177 (8)
$NaHCO_3$-P*i*	mg P kg^{-1}	4 (4)	1 (1)	6 (1)	1 (0)
Total P	mg P kg^{-1}	227 (21)	315 (22)	540 (16)	156 (9)
Total Inorganic P	mg P kg^{-1}	51 (6)	157 (11)	174 (15)	58 (4)
Total N	g N kg^{-1}	35 (1)	18 (1)	45 (0)	17 (1)
Total C	g C kg^{-1}	413 (9)	261 (11)	448 (3)	250 (9)
Microbial Biomass C	g C kg^{-1}	24 (14)	14 (2)	30 (5)	10 (3)
Microbial Biomass N	mg N kg^{-1}	1350 (85)	1290 (202)	2880 (250)	1170 (334)
Microbial Biomass P	mg P kg^{-1}	305 (65)	117 (17)	87 (22)	53 (25)
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	-	-	89 (12)	26 (3)
Aerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	53 (1)	36 (4)	-	-
Anaerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	36 (11)	34 (15)	-	-
CH_4 Production	mg C kg^{-1} d^{-1}	-	-	564 (53)	181 (23)
PMN	mg N kg^{-1} d^{-1}	-	88 (10)	212 (25)	49 (7)
PMP	mg P kg^{-1} d^{-1}	-	5 (2)	24 (8)	2 (0)
0-3 cm Soil					
Loss on Ignition	%	91 (0)	72 (3)	90 (1)	20 (2)
Extractable Organic C	g C kg^{-1}	3 (0)	3 (0)	5 (1)	2 (0)
Extractable NH_4-N	mg N kg^{-1}	116 (5)	112 (14)	109 (6)	29 (3)
$NaHCO_3$-P*i*	mg P kg^{-1}	2 (1)	1 (0)	8 (3)	1 (0)
Total P	mg P kg^{-1}	223 (10)	358 (27)	360 (10)	120 (11)
Total Inorganic P	mg P kg^{-1}	45 (3)	153 (16)	103 (7)	50 (4)
Total N	g N kg^{-1}	36 (1)	29 (2)	41 (1)	10 (1)
Total C	g C kg^{-1}	459 (7)	360 (13)	463 (3)	193 (8)
Microbial Biomass C	g C kg^{-1}	3 (1)	3 (0)	3 (1)	2 (0)
Microbial Biomass N	mg N kg^{-1}	194 (38)	294 (46)	597 (97)	317 (85)
Microbial Biomass P	mg P kg^{-1}	77 (7)	76 (8)	24 (6)	20 (3)
Soil Oxygen Demand	mg kg^{-1} hr^{-1}	14 (4)	21 (3)	33 (3)	15 (5)
Aerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	13 (5)	18 (6)	-	-
Anaerobic CO_2 Prod.	mg C kg^{-1} hr^{-1}	10 (2)	13 (3)	-	-
CH_4 Production	mg C kg^{-1} d^{-1}	-	-	105 (13)	21 (2)
PMN	mg N kg^{-1} d^{-1}	27 (3)	35 (7)	24 (4)	9 (2)
PMP	mg P kg^{-1} d^{-1}	2 (0)	4 (1)	4 (0)	2 (0)

Table 6.2. Significant relationships ($P<0.05$) among indicators of the hydrologic units based on comparisons between controls and mesocosms receiving 3.2 g P m^{-2} yr^{-1} at the final P loading period. Data for higher P loading rates are included for Water Conservation Area-2a. The PMN and PMP refer to potentially mineralizable N and P, respectively.

	P Loading Rate (g P m^{-2} yr^{-1})					
	WCA-1	WCA-3a	TS	WCA-2a		
Parameter	3.2	3.2	3.2	3.2	6.4	12.8
Floc						
Loss on Ignition	NS	NS	NS	NS	NS	NS
Extractable Organic C	NS	NS	NS	NS	NS	NS
Extractable NH_4-N	-	S	NS	NS	S	S
$NaHCO_3$-*Pi*	S	S	NS	NS	S	S
Total P	S	S	S	S	S	S
Total Inorganic P	S	NS	S	S	S	S
Total N	NS	NS	NS	NS	NS	NS
Total C	NS	NS	NS	NS	NS	NS
Microbial Biomass C	NS	S	NS	S	S	NS
Microbial Biomass N	NS	NS	S	S	S	S
Microbial Biomass P	S	S	NS	S	S	S
Soil Oxygen Demand	-	NS	NS	-	-	-
Aerobic CO_2 Prod.	NS	-	-	NS	NS	S
Anaerobic CO_2 Prod.	NS	-	-	NS	NS	S
CH_4 Production	-	NS	NS	-	-	-
PMN	-	S	S	S	S	S
PMP	-	NS	S	S	S	S
0-3 cm Soil						
Loss on Ignition	NS	NS	NS	NS	NS	NS
Extractable Organic C	NS	NS	S	NS	NS	S
Extractable NH_4-N	NS	S	NS	NS	S	S
$NaHCO_3$-*Pi*	S	NS	NS	NS	S	S
Total P	NS	NS	NS	S	S	S
Total Inorganic P	S	NS	NS	S	S	S
Total N	NS	NS	NS	NS	NS	NS
Total C	NS	NS	NS	NS	NS	NS
Microbial Biomass C	NS	NS	NS	S	S	S
Microbial Biomass N	NS	S	NS	S	S	S
Microbial Biomass P	NS	S	S	S	S	S
Soil Oxygen Demand	NS	NS	NS	S	NS	S
Aerobic CO_2 Prod.	NS	-	-	NS	NS	S
Anaerobic CO_2 Prod.	NS	-	-	NS	NS	NS
CH_4 Production	-	NS	S	-	-	-
PMN	NS	NS	NS	S	S	S
PMP	NS	NS	NS	NS	NS	NS

NS = not significant at $P< 0.05$; S = significant at $P<0.05$

enhanced by P loading. Extractable TOC was also higher($P<0.05$) in WCA-3a floc and soil than in other hydrologic units (Table 6.1).

Extractable NH_4-N was not increased by P loading in TS mesocosms and showed few changes due to duration of P loading (Table 6.2). In WCA-2a, extractable NH_4-N was increased in floc and soil at the highest P loading rates. In WCA-3a, extractable NH_4-N was enhanced at the highest P loading rate in floc and 0-3 cm soil. However, extractable NH_4-N was significantly higher in floc than in soil for all hydrologic units. Extractable NH_4-N concentrations were significantly higher in WCA-3a floc samples than at other hydrologic units, but no differences were observed in underlying soil among the various hydrologic units.

In WCA-1, $NaHCO_3$-P*i* was increased ($P<0.05$) in floc and soil but only at the highest P loading rate (Table 6.2). The $NaHCO_3$-P*i* generally contributed less than 2% of the TP in WCA-1 and contributed up to 10% of the TP in WCA-2a. In TS, $NaHCO_3$-P*i* was greater in floc than 0-3 cm soil but was not affected by P loading. WCA-3a sites generally had the highest $NaHCO_3$-P*i* concentrations.

For WCA-1, P loading significantly increased TP and TP*i* concentrations in floc after 14 months from the onset of P loading, but only at rates of 1.6 and 3.2 g m^{-2} yr^{-1} (data not shown). Even after 31 months of loading, increases in floc TP and TP*i* were only observed at the highest 2 loading rates (data not shown). In WCA-1 soil, no increases in TP or TP*i* were observed even by 31 months of loading (Table 6.2). Thus, most of the added P was present in biologically active floc sediments and a lag phase developed from the time added P had reached the underlying 0-3 cm soil.

In WCA-2a floc, significant TP increases were observed at the 3.2 g m^{-2} yr^{-1} rate by 22 months after loading, but increases in TP*i* were observed at the 1.6 g m^{-2} yr^{-1} rate after 22 months (data not shown). In WCA-2a soil, increases in TP and TP*i* were enhanced at the highest P loading rate (Table 6.2). In contrast to the WCA-1 where TP*i* was approximately 25% of TP, TP*i* contributed almost 50% of the TP in WCA-2a floc and soil.

In WCA-3a mesocosms, floc TP and TP*i* increases ($P<0.05$) were observed at the 3.2 g m^{-2} yr^{-1} rate after 4 months from the onset of P loading (data not shown). In soil, no impacts of P loading were observed after 11 months of P loading to the WCA-3a mesocosms (Table 6.2). In TS floc, TP and TP*i* increases were only observed at the highest P loading rate by 4 and 11 months after the onset of P loading. However, no changes in 0-3 cm soil TP or TP*i* were observed at any loading rate even after 11 months of P loading (Table 6.2). For all mesocosm sites in the 4 hydrologic units, floc TP and TP*i* were significantly higher than 0-3 cm soil concentrations (Table 6.1). The duration of P loading generally did not increase TP or TP*i* concentrations in floc beyond those observed by the first sampling periods.

Microbial Biomass

The MBC in WCA-1 and TS floc and 0-3 cm soil was not enhanced by P loading (Table 6.2). However, in WCA-2a, MBC was significantly increased by P loading in both floc and soil at the highest P loading rates. In WCA-3a, MBC was enhanced by P loading in floc but not in 0-3 cm soil, and MBC was up to 10 times higher in floc than in underlying soil. For all hydrologic units, MBC was significantly higher in floc than in 0-3 cm soil (Table 6.1). The floc MBC was significantly higher in WCA-3a than in other

hydrological units, but no differences among hydrological units were observed in 0-3 cm soil.

Phosphorus loading rates had little influence on MBN in floc or soil in WCA-1 mesocosms (Table 6.2). In WCA-2a floc and soil, MBN was increased after 22 months of P loading. In WCA-3a, MBN in soil increased at the highest P loading rate after 11 months (data not shown). In addition, the MBN was approximately 5 times greater in floc than in underlying soil for WCA-3a. In TS soil, MBN was not affected by P loading but was enhanced ($P<0.05$) in floc at the highest P loading rate. In addition, the MBN in TS was approximately 4 times greater in floc than in underlying 0-3 cm soil (Table 6.1).

In WCA-1, MBP was increased ($P<0.05$) after 14 months at a P loading rate of 1.6 g P m^{-2} yr^{-1} (data not shown). In WCA-2a floc and soil, MBP was increased ($P<0.05$) at a rate of 3.2 g P m^{-2} yr^{-1} (Table 6.2). In WCA-3a mesocosms, no P loading effects on MBP were observed by 4 months, but P loading significantly increased MBP after 11 months in floc and soil (Table 6.2). In addition, WCA-3a-north mesocosms showed higher ($P<0.05$) MBP than WCA-3a-south mesocosms after 11 months of P loading (data not shown). No impacts of P loading on MBP were observed in TS floc but P loading increased MBP in 0-3 cm soil (Table 6.2). For all 4 hydrologic units, MBP was significantly higher in surface floc than in underlying soil (Table 6.1). Mesocosms in WCA-1 also exhibited higher floc MBP than in other hydrologic units (Table 6.1).

Microbial Processes

The SOD and aerobic/anaerobic CO_2-production rates were not significantly impacted by P loading in WCA-1 floc and soil, but CO_2-production rates tended to be highest at the highest P loading rate and were higher ($P<0.05$) in floc than in soil (Tables

6.1 and 6.2). Aerobic and anaerobic CO_2 production rates in WCA-2a significantly increased only at the highest P loading rate of 12.8 g P m^{-2} yr^{-1} (Table 6.2). Aerobic CO_2-production rates were greater ($P<0.05$) in WCA-1 floc than in WCA-2a floc, but no differences were observed with anaerobic CO_2 production and SOD. The SOD and CH_4 production rates in WCA-3a floc and soil were not impacted by P loading but were significantly higher in floc than in underlying soil (Tables 6.1 and 6.2). In TS soil, CH_4 production rates were enhanced by P loading at the highest loading rate, but no such effects were observed in floc (Table 6.2). The SOD and CH_4 production rates were significantly highest in WCA-3a floc and soil than in other hydrologic units (Table 6.1). The SOD, CH_4, and CO_2 production rates were all significantly higher in surface floc than in the underlying 0-3 cm soil.

Phosphorus loading had little influence on PMN in soil for WCA-1 mesocosms (Table 6.2). The PMN of floc and 0-3 cm soil was significantly increased by P loading in WCA-2a, particularly at higher P loading rates (Table 6.2). The PMN was significantly enhanced in WCA-3a and TS floc but not in underlying 0-3 cm soil (Table 6.2). In addition, PMN was significantly higher in floc than in soil for both WCA-3a and TS mesocosms. The PMN in WCA-3a floc was higher ($P<0.05$) than in other hydrologic units and generally lowest in TS floc and soil (Table 6.1).

In WCA-2a floc, PMP was increased at the 3.2 g m^{-2} yr^{-1} rate (Table 6.2). Phosphorus loading also enhanced PMP rates in TS floc but not in WCA-3a mesocosms. The PMP rates were significantly higher in WCA-3a than in other hydrologic units, but only in the floc (Table 6.1). No differences in PMP among the various units were

observed in underlying soil (Table 6.2). The PMP was also significantly higher for floc than for 0-3 cm soil in WCA-3a but not in other hydrologic units (Table 6.1).

Relationships between Biogeochemical Indicators and Microbial Processes

Microbial biomass C was significantly correlated with extractable TOC (Tables 6.3 and 6.4), with approximately 50% of the MBC variability being explained by extractable TOC, NH_4-N, and TP*i* (Table 6.5). The MBN was significantly correlated with extractable NH_4-N and TN concentrations and with various heterotrophic processes such as SOD, CH_4 production, and CO_2-production rates (Tables 6.3 and 6.4). Significant relationships were observed between MBP and various floc and soil P parameters (Tables 6.3 and 6.4). The MBP appeared to be controlled by TN, extractable NH_4-N, $NaHCO_3$-P*i*, and TP (Tables 6.3 and 6.4), with 84% of the MBP variability being explained (Table 6.5).

The SOD and CH_4-production rates were significantly correlated with extractable TOC and NH_4-N (Tables 6.3 and 6.4). Significant relationships were observed between NH_4-N concentrations and the CH_4 production rates in both floc and soil (Fig. 6.1).

The PMN rates were a measure of the potential organic N mineralization rates. The PMN rates were also significantly related to SOD, CH_4, and CO_2-production rates, providing a linkage between organic C and N mineralization and cycling in soils. In addition, TP and MBC appeared to be controlling factors of both SOD and CH_4-production rates (Table 6.5). Microbial biomass C, N, and P appeared to be regulating factors of heterotrophic microbial activities and PMN and PMP rates in floc and soil. In addition, the P fractions in floc and soil appeared to be major regulating factors of microbial activities. The PMN rate was significantly correlated with extractable TOC

Table 6.3. Significant correlation coefficients (P <0.05) among indicators of Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough floc (n = 195)

Parameter	LOI	Ext. TOC	NH_4-N	$NaHCO_3$-P*i*	TP	TP*i*	TN	TC	MBC	MBN	MBP	PMN	PMP	SOD
Loss on Ignition		0.56	0.64						0.50	0.49		0.73	0.41	
Extractable NH_4-N		0.71							0.67	0.58				
$NaHCO_3$-P*i*											0.57	0.37		
Total P			0.41	0.66					0.46	0.34	0.76	0.63	0.68	
Total Inorganic P				0.74	0.93						0.76	0.45	0.70	
Total N	0.97	0.56	0.66						0.49	0.48		0.71	0.42	
Total C	0.98	0.58	0.65				0.97		0.50	0.48		0.70	0.40	
Microbial Biomass C		0.51												
Microbial Biomass N		0.62							0.49					
Microbial Biomass P									0.30			0.40		
Soil Oxygen Demand	0.72	0.53	0.58	0.38	0.71	0.56	0.71	0.72	0.45	0.41	0.48	0.58	0.37	
Aerobic CO_2 Prod.			0.43	0.46						0.42		0.44		
CH_4 Production	0.71	0.80	0.90		0.61	0.38	0.70	0.71	0.73	0.59	0.58	0.68		0.74
PMN		0.42	0.47						0.53	0.56				
PMP				0.51							0.43	0.62		

Table 6.4. Significant correlation coefficients ($P<0.05$) among indicators of Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough 0-3 cm soil (n = 195).

Parameter	LOI	Ext. TOC	NH_4-N	$NaHCO_3$-P*i*	TP	TP*i*	TN	TC	MBC	MBN	MBP	PMN	PMP	SOD	Aerobic
Extractable NH_4-N		0.41													
$NaHCO_3$-P*i*		0.34								0.35		0.40			
Total P	0.44			0.54							0.41	0.51	0.60		
Total Inorganic P				0.53							0.39	0.41	0.76		
Total N	0.95	0.47	0.62		0.42										
Total C	0.98	0.40	0.63		0.38		0.96								
Microbial Biomass N		0.44													
Microbial Biomass P			0.41									0.74			
PMN		0.33	0.38						0.47	0.40					
PMP				0.48											
Soil Oxygen Demand	0.53	0.38	0.41	0.48	0.63	0.49	0.48	0.51				0.44			
Aerobic CO_2 Prod.										0.41	0.44	0.35		0.61	
Anaerobic CO_2 Prod.									0.35					0.50	0.58
CH_4 Production	0.69	0.73	0.63	0.37	0.81	0.77	0.68	0.68	0.34	0.74	0.66	0.92	0.78	0.63	

Table 6.5. Significant regression equations ($P<0.05$) for floc and 0-3 cm soil for Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough. The MBC = microbial biomass C; MBN = microbial biomass N; MBP = microbial biomass P; SOD = soil oxygen demand; CH_4 = methane production rate; PMN = potentially mineralizable N; PMP = potentially mineralizable P; TP*i* = total inorganic P; TP = total P; LOI = loss on ignition.

Regression Equation	R^2
Floc (n = 195)	
MBC = -5900 + 0.8 (ext. TOC) + 70 (NH_4-N) + 24 (TP*i*)	0.51
MBN = -159 + 0.2 (ext. TOC) + 1.6 (NH_4-N) + 0.9 (TP)	0.45
MBP = 6.8 - 6.0 (TN) + 0.3 (NH_4-N) + 0.9 ($NaHCO_3$-P*i*) + 0.5 (TP)	0.84
SOD = -5.1 + 0.003 (ext. TOC) + 0.1 (TP)	0.56
CH_4 = -26 + 0.01 (ext. TOC) + 0.6 (NH_4-N) + 0.002 (MBC)	0.88
PMN = -39 + 2.2 (LOI) + 0.1 (TP*i*)	0.66
PMP = -17 + 0.5 (LOI) - 0.0005 (MBC) + 0.1 (TP*i*)	0.72
0-3 cm Soil (n = 195)	
SOD = 2.5 + 0.001 (ext. TOC) + 0.1 (TP)	0.42
CH_4 = -65 + 0.007 (ext. TOC) + 0.008 (MBC) + 0.3 (TP)	0.76
PMN = 2.3 + 0.02 (MBN) + 0.3 (MBP)	0.64
PMP = 0.3 - 0.1 (TP) - 0.0005 (MBC) + 0.2 (TP*i*)	0.62

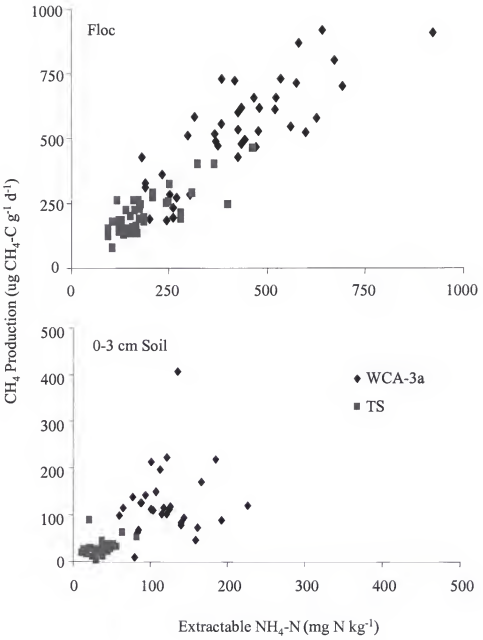

Figure 6.1. The CH_4-production rates in floc and 0-3 cm soil of mesocosms in Water Conservation Area-3a (WCA-3a) and Taylor Slough as related to extractable NH_4-N.

and NH_4-N and with microbial biomass C and N (Tables 6.3 and 6.4). The PMP was significantly correlated with MBP, $NaHCO_3$-P*i*, and TP (Tables 6.3 and 6.4), and approximately 63% of the PMP variability was explained with these parameters (Table 6.5).

Impact Index

An impact index was developed to eliminate the units of various biogeochemical indicators so that they may be expressed on a similar basis to allow for comparisons to be made between indicators (Table 6.6). The formula is:

Impact Index = log [(P-impacted area) / (reference area)].

The LOI, extractable TOC, TN, and TC showed relatively little impact of P loading while the P-related parameters, particularly $NaHCO_3$-P*i*, were most strongly affected by P loading (Table 6.6). The most responsive microbial process to P loading was PMP in both floc and 0-3 cm soil while other microbial processes were less responsive to P loading. Thus, the P-related indicators were most sensitive to P loading and best reflected changes in soil biogeochemical processes after P loading. A relative greater impact of P loading was observed on MBP than on MBC or MBN in floc samples for the various hydrologic units (Table 6.6). However, in underlying soil, the microbial biomass exhibited little impacts due to P loading in WCA-1, WCA-2a, and TS. Only for the high P-dosed WCA-2a mesocosms did microbial biomass show impacts as a result of P loading.

Discussion

Phosphorus loading increased TP, $NaHCO_3$-P*i*, and TPi in surface floc sediments of mesocosms, with significant increases being most readily observed at the highest P

Table 6.6. An impact index [log (P-impacted area/reference area)] for various biogeochemical indicators in floc and soil for Water Conservation Area-1 (WCA-1), WCA-2a, WCA-3a, and Taylor Slough at the last the P-loading period. The PMN and PMP refer to potentially mineralizable N and P, respectively.

	P Loading Rate (g P m^{-2} yr^{-1})					
	WCA-1	WCA-3a	TS	WCA-2a		
Parameter	3.2	3.2	3.2	3.2	6.4	12.8
Floc						
Loss on Ignition	0.0	0.0	0.1	-0.1	0.0	0.0
Extractable Organic C	-0.2	-0.1	0.1	0.0	0.0	0.0
Extractable NH_4-N	-	0.1	0.2	0.1	0.2	0.1
$NaHCO_3$-*Pi*	1.7	0.3	0.2	0.0	1.5	2.0
Total P	0.4	0.2	0.3	0.4	0.6	0.6
Total Inorganic P	0.7	0.3	0.4	0.5	0.7	0.7
Total N	0.0	0.0	0.1	-0.1	0.0	0.0
Total C	0.0	0.0	0.0	0.0	0.0	0.0
Microbial Biomass C	-0.5	-0.2	0.1	0.1	0.3	0.0
Microbial Biomass N	-0.2	-0.1	0.2	0.2	0.2	0.2
Microbial Biomass P	0.4	0.2	0.5	0.6	0.6	0.9
Soil Oxygen Demand	-	0.1	0.2	-	-	-
Aerobic CO_2 Prod.	0.2	-	-	0.0	-0.2	0.2
Anaerobic CO_2 Prod.	0.1	-	-	0.1	0.1	0.2
CH_4 Production	-	-0.1	0.2	-	-	-
PMN	-	-0.1	0.3	0.2	0.3	0.4
PMP	-	0.3	0.5	1.1	1.5	1.5
0-3 cm Soil						
Loss on Ignition	0.0	0.0	0.0	-0.1	-0.1	0.0
Extractable Organic C	0.0	0.0	0.0	0.0	0.0	0.1
Extractable NH_4-N	0.2	0.2	0.2	0.2	0.2	0.3
$NaHCO_3$-*Pi*	0.3	-0.1	-0.3	0.5	1.1	1.5
Total P	0.0	0.0	-0.1	0.1	0.2	0.1
Total Inorganic P	0.1	-0.1	-0.1	0.2	0.3	0.2
Total N	0.0	0.0	-0.1	0.0	-0.1	0.0
Total C	0.0	0.0	0.0	0.0	0.0	0.0
Microbial Biomass C	0.1	0.0	0.0	0.3	0.3	0.2
Microbial Biomass N	-0.1	0.1	0.0	0.3	0.4	0.4
Microbial Biomass P	0.1	0.0	0.1	0.2	0.3	0.3
Soil Oxygen Demand	0.0	0.0	-0.1	0.1	0.1	0.3
Aerobic CO_2 Prod.	-0.2	-	-	0.0	0.0	0.3
Anaerobic CO_2 Prod.	0.0	-	-	0.0	0.1	0.2
CH_4 Production	-	-0.2	0.1	-	-	-
PMN	0.0	0.0	0.0	0.5	0.3	0.4
PMP	0.2	-0.1	0.0	0.4	0.5	0.5

loading rates (Table 6.2). Mass P loading had limited impacts on floc P parameters, as most parameter increases were observed by the first sampling period and subsequent mass loading of P produced only a few significant responses that were not observed by the first sampling period. An exception occurred for WCA-2a, where the mass loading of P was several orders of magnitude higher than for other hydrologic units. The more calcareous nature of WCA-2a mesocosms likely led to increased P fixation and subsequent increased TP*i* compared to the WCA-1 mesocosms.

The TP and TP*i* were significantly higher in floc than in 0-3 cm soil. Soil P accumulation below the surface floc layer may take years to develop, depending on the P loading rate (McCormick et al., 1996). Many parameters in the underlying 0-3 cm soil were not impacted by P loading. Most of the added P may have been fixed or adsorbed to floc sediments, and thus the underlying soil showed little increase in TP, $NaHCO_3$-P*i*, or TP*i*. Hence, microbial biomass and various microbial processes exhibited delayed or diminished response to added P in the 0-3 cm soil. Only at the highest loading rates, particularly in WCA-2a, were significant responses of microbial biomass in soil observed due to P loading.

For TS (initial floc TP of approximately 100-200 mg P kg^{-1}), the mass P loading at the highest rate after 5 months was 1.33 g P m^{-2}. This rate produced significant increases in floc TP concentrations by 50-100% and significant increases in various microbial processes (data not shown). Likewise, mass P loading for the highest P-loading rate for WCA-3a (initial floc TP of approximately 400-600 mg P kg^{-1}) after 4 months (1.07 g P m^{-2}) only produced increases in floc TP of approximately 20% (data not shown). After 11 months after mass loading of 2.93 g P m^{-2} to WCA-3a, floc TP

increased approximately 50-100% (data not shown). In WCA-1, significant ($P<0.05$) increases in floc TP were observed at a mass loading of 1.86 g P m^{-2} after 14 months (data not shown). In WCA-2a, significant increases in floc TP were observed at the P loading rate of 3.2 g P m^{-2} yr^{-1} or a mass loading of 5.86 g P m^{-2} (data not shown). This evidence suggests that the impacts of P loading on retention of added P and subsequent response of various microbial parameters or processes is dependent upon the initial TP concentration of the floc. Mesocosm with the lowest initial TP floc concentrations, such as in TS, showed significant responses to added P at a lower mass P loading than sites having higher initial floc P concentrations, such as in WCA-2a.

The most responsive parameters to P loading were P-related, such as $NaHCO_3$-P*i*, TP, TP*i*, MBP, and PMP (Table 6.6). The mesocosms were established in oligotrophic areas of the respective hydrologic units; thus, P was considered to be the limiting nutrient in these systems. Thus, P loading significantly increased various P parameters. However, significant increases in many parameters were only observed at the highest P loading rates, suggesting that biotic communities in floc sediments have a capacity to assimilate low levels of added P with minimal changes in microbial biomass or processes.

The P-loading effects on microbial biomass and related processes were more readily observed in surface floc sediments than in underlying soil. Based on such results, sustained long-term high P loading, such as observed in the WCA-2a, was required to induce major changes in microbial biomass and organic matter degradation (Wright and Reddy, 2001b). At lower P dosing rates, the added P may become rapidly assimilated by biotic communities in floc sediments or immobilized through precipitation with calcium

minerals. This may explain the lack of microbial response to added P at the lower P loading rates.

Microbial activity measurements, such as SOD, CO_2 production, and CH_4 production were significantly related to NH_4-N, suggesting that a NH_4-N limitations may negatively impact heterotrophic microbial activity. Indeed, N limitations were observed in P-impacted areas of the Everglades (White and Reddy, 2000). Similar relationships between CH_4 and NH_4-N were observed in Everglades soil (Koch-Rose et al., 1994). Ammonium is likely mineralized from soil organic matter through both aerobic and anaerobic degradation pathways; thus, both SOD and CH_4-production rates were significantly correlated with NH_4-N in both floc and underlying soil. Microbial CO_2 and CH_4-production rates were also enhanced at P-impacted sites near water inflow points or canals in the various hydrologic units (Davis, 1991; DeBusk and Reddy, 1998), though increases were observed at higher TP concentrations than developed at mesocosm P loading rates (Wright and Reddy, 2001b). Increases in NH_4-N concentrations at high P loading rates in various hydrologic units may be due to stimulation of organic matter degradation and subsequent nutrient release (McCormick et al., 2000; Newman et al., 2001). Also, as the P limitations of floc and soil are eliminated by P loading, other nutrients, particularly NH_4-N, may become limiting (White and Reddy, 2000; Newman et al., 2001). Hence, NH_4 released from organic matter degradation should tend to enhance heterotrophic microbial activities. Newman et al. (2001) showed that NH_4 accumulation in soils was a sensitive indicator of P loading, because P stimulated organic matter degradation and nutrient regeneration. In addition, NH_4 may be able to serve as an

electron donor for anaerobic microorganisms, thus CH_4-production rates and NH_4-N accumulation would be correlated.

The four hydrologic units in this study received various P loading rates for different time periods. The hydrologic units also differed in their substrate composition. The WCA-2a and TS mesocosms were established in organic soils having substantial amounts of $CaCO_3$ interspersed, while WCA-1 and WCA-3a soils were primarily organic in nature (McCormick et al., 2000). In addition, these hydrologic units had initially variable background TP concentrations (Table 6.1). However, the biogeochemical indicators of the various hydrologic units exhibited similar responses to low-level P loading. However, the addition of P to TS, which had the lowest background P concentrations, promoted changes in microbial biomass and related processes not observed in other hydrologic units receiving the same P loading rates. Hence, the background P concentrations of hydrologic units are likely to serve as controlling factors for potential impacts of P loading on microbial biomass and processes. Indeed, soil P concentrations have proved to be controlling factors of decomposition rates in Everglades soils (DeBusk and Reddy, 1998; Wright and Reddy, 2001b).

Related studies in the four hydrologic units along several transects that encompassed both P-impacted sites near inflow waters and interior sites not impacted by P showed that microbial parameters and processes were enhanced by P loading (Wright and Reddy, 2001b). In addition to gradients in P concentrations, transects encompassed different vegetative zones. *Typha* was dominant in areas adjacent to inflow points, while *Cladium* dominated the interior of the hydrologic units (Davis, 1991). Transects have also been exposed not only to P loading but to external loading of inorganic and organic

N and S. These transect sites have also been subjected to nutrient loading over a longer time period and have substantially higher floc and soil TP concentrations than observed in the mesocosms. Thus, the magnitude of microbial responses to nutrient loading in transects generally was greater than those observed in mesocosms. However, the relationships between P, microbial biomass, and associated processes was consistent between transect sites and mesocosms (Wright and Reddy, 2001b). Hence, changes in microbial biomass and related processes observed along the nutrient gradients were most likely due to P loading rather than to differences in vegetation or N loading.

Conclusions

Impacts of P dosing on chemical and microbial parameters in various hydrologic units of the Everglades were readily observed but primarily in surface floc sediments and at the highest P loading rates. Some P-unimpacted sites within various hydrologic units may have an ability to assimilate low P loading with minimal changes in soil microbial biomass, heterotrophic microbial activity, or organic matter mineralization rates.

Phosphorus loading increased floc TP rates but generally only at the highest P loading rates. Phosphorus loading had little or no influence on the underlying soil, suggesting development of a lag phase between P loading and impacts on soil processes. Even after 3 years of P loading, few significant changes in soil biogeochemical indicators were observed in underlying soil. Only at high P loading rates will impacts be observed in underlying soil. Uptake or accumulation of added P by floc and algal sediments was primarily responsible for the removal of added inorganic $NaHCO_3$-P*i* from floodwater. It was in this component that most of the effects of P loading were observed. An undesirable impact of P loading was the stimulation of heterotrophic microbial activity

and organic matter degradation which has likely resulted in the regeneration of inorganic nutrients to the floodwater. Phosphorus loading enhanced microbial biomass, thus stimulating heterotrophic microbial activities and organic matter degradation, as observed by CO_2 and CH_4 production, PMN, and PMP rates.

Lower mass loading of P into hydrologic units of the Everglades which have been minimally impacted by external P loading, such as TS, produced a greater response by biogeochemical indicators than hydrologic units exhibiting higher background P levels, such as WCA-2a. Wetlands with initially low TP were more susceptible to alterations in microbial parameters after the onset of P loading than were sites exhibiting higher TP levels. Thus the various hydrologic units, particularly those in southern areas or non-impacted interior areas with lower TP levels, are more sensitive to P loading.

CHAPTER 7
CONCLUSIONS

Laboratory and field studies were conducted to determine the impacts of nutrient loading on microbial processes in Everglades soils. Microbial processes investigated included extracellular enzyme activity (Chapter 2) and heterotrophic microbial activities such as carbon dioxide (CO_2) and methane (CH_4) production (Chapter 3). The utilization of various types of electron donors and inorganic nutrients by soil microorganisms under various levels of P enrichment was determined (Chapter 4). The impacts of nutrient loading, particularly phosphorus (P), on soil biogeochemical indicators of four hydrologic units of the Everglades were studied along various P gradients (Chapter 5). In addition, the impacts of experimental P dosing to oligotrophic wetland soils on various biogeochemical indicators were investigated in four hydrologic units of the Everglades (Chapter 6). A summary of experimental results related to experimental objectives is presented below.

1) Determine the impacts of nutrient loading on extracellular enzyme activity in detritus and soil. Develop relationships between extracellular enzyme activities and various soil biogeochemical indicators

Five extracellular enzyme activities were measured along a nutrient gradient in WCA-2a, including alkaline phosphatase, arylsulfatase, glucosidase, protease, and phenol oxidase. Alkaline phosphatase activity (APA) increased with increasing distance from the primary water inflow point and was inversely related to soil P

parameters. Other enzyme activities were not influenced by P loading and showed few relationships with other biogeochemical indicators. The APA was significantly negatively related to P loading and to various soil P properties, and thus proved to be a sensitive indicator of P enrichment. All enzyme activities were highest in surface detritus and decreased with increasing soil depth, corresponding to decreases of microbial biomass with depth.

2) Determine the impacts of nutrient loading, electron acceptors, electron donors, and inorganic nutrients on heterotrophic microbial activities in various hydrologic units of the Everglades. Relationships between various microbial processes and soil biogeochemical indicators will also be developed.

Heterotrophic microbial activities were assessed by measuring CO_2 and CH_4-production rates. Heterotrophic microbial activities were highest in P-impacted areas near inflow waters of the various hydrologic units, and decreased with increasing distance from the inflow. Seasonal influences on heterotrophic microbial activity were minimal. Additions of various inorganic electron acceptors enhanced heterotrophic microbial activity in both P-impacted and P-unimpacted areas. Denitrification and SO_4 reduction rates were approximately 30-40% of aerobic respiration rates. Meanwhile, CH_4-production rates were generally less than 10% of aerobic respiration rates. Thus, water management in the Everglades is important in regulating heterotrophic microbial activity and organic matter degradation. Low water levels have the potential for rapid aerobic organic matter decomposition rates and subsequent nutrient regeneration, thus increasing inorganic N and P concentrations in the water column.

Heterotrophic microbial activities were highest in surface detritus and decreased with soil depth. Surface detritus was more sensitive to P loading and to additions of electron acceptors and donors, because this material consists of recently deposited plant matter and benthic periphyton, and supports higher microbial biomass.

Additions of various electron donors enhanced heterotrophic microbial activity in both P-impacted and unimpacted reference areas, suggesting that labile organic carbon (C) was limiting heterotrophic microbial activity in these soils. Utilization rates of electron donors were greater in surface detritus than in underlying soil. Soil microorganisms of the Everglades exhibited a wide capability to degrade various alcohols, amides, amino acids, aromatics, carboxylic acids, and polysaccharides. Additions of inorganic nutrients such as ammonium (NH_4-N) and phosphate enhanced heterotrophic microbial activity in oligotrophic soils but not in the P-impacted soils, thus showing that bioavailable nutrients may limit heterotrophic microbial activity in interior areas of the hydrologic units. Thus, continued external P loading into interior areas of hydrologic may further enhance heterotrophic microbial activities, leading to increased organic matter degradation and nutrient cycling.

3) Develop soil biogeochemical indicators of P enrichment in various hydrologic units of the Everglades. Determine which indicators are sensitive to P loading. Investigate differences in biogeochemical indicators between various hydrologic units in their response to nutrient loading.

Many biogeochemical indicators were measured in four hydrologic units of the Everglades. Various indicators, such as total P, total inorganic P, and $NaHCO_3$-P*i*,

were the most sensitive indicators of P enrichment for all four hydrologic units, with these parameters being significantly increased by P loading. Microbial biomass P and potentially mineralizable P were somewhat less sensitive than other P related physico-chemical parameters, but still showed significant relationships to P loading. Thus, the P-related biogeochemical indicators were excellent indicators of P enrichment in Everglades soils.

Microbial biomass C and N were correlated with extractable TOC and NH_4-N in addition to various P parameters; thus, microbial biomass appeared to be controlled by extractable TOC and NH_4-N in addition to P. Hence, microbial biomass C and N were not sensitive indicators of P enrichment. Likewise, various microbial processes, including CO_2 and CH_4-production rates, in addition to potentially mineralization N (PMN), appeared to be controlled by microbial biomass in addition to extractable nutrients. Thus, only biogeochemical indicators directly related to P loading, such as P-related indicators, were sensitive to P loading and thus would serve as adequate indicators of P enrichment.

Some differences in biogeochemical indicators between the various hydrologic units were observed. Low P loading had a more significant effect on biogeochemical indicators in soils initially low in P, such as for Taylor Slough. Hydrologic units which exhibited higher TP levels, such as for WCA-2a, were less sensitive to P loading. Thus, potential impacts of P loading to Everglades soils were dependent on the initial background soil TP concentrations. Future P loading into the Everglades will potentially have a greater impact on soil biogeochemical indicators in hydrologic units low exhibiting low P levels, such as Taylor Slough.

REFERENCES

Achtnich, C., F. Bak, and R. Conrad. 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. Biol. Fert. Soils 19:65-72.

Amador, J. A., and R. D. Jones. 1993. Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. Soil Biol. Biochem. 25:793-801.

Amador, J. A., and R. D. Jones. 1995. Carbon mineralization in pristine and phosphorus-enriched peat soils of the Florida Everglades. Soil Sci. 159:129-141.

Anderson, J. M. 1976. An ignition method for determination of total phosphorus in lake sediments. Water Res. 10:329-331.

Bachoon, D., and R. D. Jones. 1992. Potential rates of methanogenesis in sawgrass marshes with peat and marl soils in the Everglades. Soil Biol. Biochem. 24:21-27.

Benner, R., A. E. Maccubbin, and R. E. Hodson. 1984. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora. Appl. Environ. Microbiol. 47:998-1004.

Billen, G. 1982. Modeling the processes of organic matter degradation and nutrient recycling in sedimentary systems. p.15-52. *In* D. B. Nedwell and C. M. Brown (eds.) Sediment Microbiology. Academic Press, London.

Boon, P. I., and B. K. Sorrell. 1991. Biogeochemistry of billabong sediments. The effect of macrophytes. Freshwater Biol. 26:209-226.

Bremner, J. M. and C. S. Mulvaney. 1982. Nitrogen-total. p. 595-624. *In* A. L. Page (ed.) Methods of Soil Analysis. Part 2. Soil Sci. Soc. Am., Madison, WI.

Bridgham, S. D., and C. J. Richardson. 1992. Mechanisms controlling soil CO_2 and CH_4 in southern peatlands. Soil Biol. Biochem. 24:1089-1099.

Brookes, P.C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soils. Soil Biol. Biochem. 17:83-842.

Cembella, A. D., N. J. Antia, and P. J. Harrison. 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective. Part 1. CRC Crit. Rev. Microbiol. 10:317-391.

Chiang, C., C. B. Craft, D. W. Rogers, and C. J. Richardson. 2000. Effects of 4 years of nitrogen and phosphorus additions on Everglades plant communities. Aquatic Bot. 68:61-78.

Chrost, R. J. 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. p. 29-59. *In* R. J. Chrost (ed.) Microbial Enzymes in Aquatic Environments. Springer-Verlag, New York, NY.

Chrost, R. J., and H. Rai. 1993. Ectoenzyme activity and bacterial secondary production in nutrient-impoverished and nutrient-enriched freshwater mesocosms. Microb. Ecol. 25:131-150.

Chua, T. T. 2000. Mineralization of organic phosphorus in a subtropical freshwater wetland. Ph. D. dissertation, Univ. of Florida, Gainesville.

Clymo, R. S. 1983. Peat. p.159-224. *In* A. J. P. Gore (ed.) Mires: Swamp, Bog, Fen, and Moor. Elsevier Publ., Amsterdam.

Condron, L. M., K. M. Goh, and R. H. Newman. 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. Soil Sci. 36:199-207.

Cotner, J. B., and R. G. Wetzel. 1991. Bacterial phosphatases from different habitats in a small, hardwater lake. p. 187-205. *In* R. J. Chrost (ed.) Microbial Enzymes in Aquatic Environments. Springer Verlag, New York, NY.

Craft, C. B., and C. J. Richardson. 1993. Peat accretion and phosphorus accumulation along a eutrophication gradient in the northern Everglades. Biogeochemistry 22:133-156.

Craft, C. B., and C. J. Richardson. 1995. Response of Everglades plant communities to nitrogen and phosphorus additions. Wetlands 15:258-271.

D'Angelo, E. M., and K. R. Reddy. 1994. Diagenesis of organic matter in a wetland receiving hypereutrophic lake water. II. Role of inorganic electron acceptors in nutrient release. J. Environ. Qual. 23:937-943.

D'Angelo, E. M., and K. R. Reddy. 1999. Regulators of heterotrophic microbial potentials in wetland soils. Soil Biol. Biochem. 31:815-830.

Davis, J. H. 1943. The natural features of southern Florida. Florida Geol. Survey, Bull. No. 25.

Davis, S. M. 1991. Growth, decomposition, and nutrient retention of *Cladium jamaicense* Crantz and *Typha domingensis* Pers. in the Florida Everglades. Aquatic Bot. 40:203-224.

DeBusk, W. F. 1996. Organic matter turnover along a nutrient gradient in the Everglades. Ph. D. Dissertation, Univ. of Florida, Gainesville.

DeBusk, W. F., S. Newman, and K. R. Reddy. 2001a. Spatio-temporal patterns of soil phosphorus enrichment in Everglades WCA-2a. J. Environ. Qual. 30:1438-1446.

DeBusk, W. F., and K. R. Reddy. 1998. Turnover of detrital organic carbon in a nutrient-impacted Everglades marsh. Soil Sci. Soc. Am. J. 62:1460-1468.

DeBusk, W. F., K. R. Reddy, M. S. Koch, and Y. Wang. 1994. Spatial distribution of soil nutrients in a northern Everglades marsh: Water Conservation Area-2a. Soil Sci. Soc. Am. J. 58:543-552.

DeBusk, W. F., J. R. White, and K. R. Reddy. 2001b. Carbon and nitrogen dynamics in wetland soil. p. 27-53. *In* M. J. Shaffer, L. Ma, and S. Hansen (eds.) Modeling Carbon and Nitrogen Dynamics for Soil Management. CRC Press, Boca Raton, FL.

Degens, B. P. 1998. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. Soil Biol. Biochem. 30:1981-1988.

Degens, B. P. 1999. Catabolic response profiles differ between microorganisms grown in soils. Soil Biol. Biochem. 31:475-477.

Degens, B. P., and J. A. Harris. 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol. Biochem. 29:1309-1320.

Degens, E. T., and K. Mopper. 1976. Factors controlling the distribution and early diagenesis of organic material in marine sediments. p. 59-113. *In* J. P. Riley and R. Chester (eds.) Chemical Oceanography. Academic Press, London.

DeGobbis, D., E. Homme-Maslowska, A. A. Orio, R. Donazzolo, and B. Pavoni. 1984. The role of alkaline phosphatase in the sediments of Venice Lagoon on nutrient regeneration. Estuar. Coast. Shelf Sci. 22:425-437.

Drake, H. L., N. G. Aumen, C. Kuhner, and C. Wagner, A. Griebhammer, and M. Schmittroth. 1996. Anaerobic microflora of Everglades sediments: effects of nutrients on population profiles and activities. Appl. Environ. Micro. 62:486-493.

Eivazi, F., and M. A. Tabatabai. 1977. Phosphatases in soils. Soil Biol. Biochem. 9:167-172.

Eivazi, F., and M. A. Tabatabai. 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20:601-606.

Fenchel, T. M., and B. B. Jorgenson. 1977. Detritus foodchains of aquatic ecosystems: the role of bacteria. Adv. Microb. Ecol. 1:1-58.

Gage, M. A., and E. Gorham. 1985. Alkaline phosphatase activity and other cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. Freshwater Biol. 15:227-233.

Godshalk, G. L., and R. G. Wetzel. 1978. Decomposition of aquatic angiosperms. II. Particulate components. Aquat. Bot. 5:301-327.

Halemejko, G. Z., and R. J. Chrost. 1984. The role of phosphatases in phosphorus mineralization during decomposition of lake phytoplankton blooms. Arhiv. Fur Hydrobiologie 101:489-502.

Happell, J. D., and J. P. Chanton. 1993. Carbon remineralization in a north Florida swamp forest: effects of water level on the pathways and rates of soil organic matter decomposition. Global Biogeochem. Cycles 7:475-490.

Harrison, P. G., and K. H. Mann. 1975. Detritus formation from eelgrass: The relative effects of fragmentation, leaching, and decay. Limnol. Oceano. 20:924-934.

Heal, O. W., P. W. Flanagan, D. D. French, and S. F. MacLean. 1981. Decomposition and accumulation of organic matter. p. 587-633. *In* L. C. Bliss (ed.) Tundra Ecosystems: a Comparative Analysis. Cambridge University Press, Cambridge.

Hedley, M.J., and J. W. B. Stewart. 1982. Method to measure microbial phosphate in soils. Soil Biol. Biochem. 14:377-385.

Hitzl, W., M. Henrich, M. Kessel, H. Insam. 1997. Application of multivariate analysis of variance related techniques in soil studies with substrate utilization tests. J. Micro. Methods 30:81-89.

Hoppe, H. G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl substrates. Mar. Ecol. Progress Ser. 11:299-308.

Hoppe, H., S. Kim, and K. Gocke. 1988. Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. Appl. Environ. Micro. 54:784-790.

Howarth, R. W., and J. M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnol. Oceano. 24:999-1013.

Ivanoff, D. B., K. R. Reddy, and S. Robinson. 1998. Chemical fractionation of organic P in histosols. Soil Sci. 163:36-45.

Jackson, C. R., C. M. Foreman, and R. L. Sinsabaugh. 1995. Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie coastal wetland. Freshwater Biol. 34:329-342.

Juma, N. G., and M. A. Tabatabai. 1978. Distribution of phosphomonoesterases in soils. Soil Science 126:101-108.

King, G. M., and M. J. Klug. 1980. Sulfhydrolase activity in sediments of Wintergreen Lake, Kalamazoo County, Michigan. Appl. Environ. Micro. 39:950-956.

Koch, M. S., and K. R. Reddy. 1992. Distribution of soil and plant nutrients along a trophic gradient in the Florida Everglades. Soil. Sci. Soc. Am. J. 56:1492-1499.

Koch-Rose, M.S., K. R. Reddy, and J. P. Chanton. 1994. Factors controlling seasonal nutrient profiles in a subtropical peatland of the Florida Everglades. J. Environ. Qual. 23:526-533.

Kuo, S. 1996. Phosphorus. p. 869-919. *In* J. M. Bingham (ed.) Methods of Soil Analysis. Part 3. Soil Sci. Soc. Am., Madison, WI.

Ladd, J. N., and J. H. A. Butler. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4:19-30.

Ladd, J. N., and R. B. Jackson. 1982. Biochemistry of ammonification. p. 173-228. *In* F. H. Stevenson (ed.) Nitrogen in Agricultural Soils. Am. Soc. Agron., Madison, WI.

Lodge, D. J., W. H. McDowell, and C. P. McSwiney. 1994. The importance of nutrient pulses in tropical forests. Tree 9:384-387.

Lovley, D. R., and M. J. Klug. 1982. Intermediary metabolism of organic matter in the sediments of a eutrophic lake. Appl. Environ. Micro. 43:552-560.

Lovley, D. R., and M. J. Klug. 1986. Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments. Geochem. Cosmo. Acta. 50:11-18.

Maltby, E., and P. J. Dugan. 1994. Wetland ecosystem protection, management, and restoration: an international perspective. p. 29-46. *In* S. M. Davis and J. C. Ogden (eds.) Everglades: The Ecosystem and its Restoration. St. Lucie Press, FL.

Marsden, W. L., and P. P. Gray. 1986. Enzymatic hydrolysis of cellulose in lignocellulose materials. CRC Crit. Rev. Biotech. 3:235-276.

Martens, R. 1987. Estimation of microbial biomass in soil by the respiration method: importance of soil pH and flushing methods for the measurement of respired CO_2. Soil Biol. Biochem. 19:77-81.

Martens, R. 1995. Current methods for measuring microbial biomass C in soils: potentials and limitations. Biol. Fert. Soils 19:87-99.

Mason, H. S. 1948. The chemistry of melanin III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. J. Biol. Chem. 172:83-99.

Mayer, S. 1989. Extracellular proteolytic enzyme activity in sediments of an intertidal mudflat. Limnol. Oceano. 34:973-981.

McCormick, P. V., S. Newman, S. L. Miao, K. R. Reddy, D. Gawlik, C. Fritz, T. D. Fontaine, and D. Marley. 2000. Ecological needs of the Everglades. Ch. 3. p.3-37. *In* Everglades Interim Report, South Florida Water Management District, West Palm Beach, FL.

McCormick, P. V., and M. B. O'Dell. 1996. Quantifying periphyton responses to P enrichment in the Florida Everglades: a synoptic-experimental approach. J. N. Am. Benth. Soc. 15:450-468.

McCormick, P. V., P. S. Rawlik, K. Lurding, E. P. Smith, and F. H. Sklar. 1996. Periphyton water quality relationships along a nutrient gradient in the northern Everglades. J. N. Am. Benth. Soc. 15:433-449.

McKinley, V. L., and J. R. Vestal. 1992. Mineralization of glucose and lignocellulose by four arctic freshwater sediments in response to nutrient enrichment. Appl. Environ. Microb. 58:1554-1563.

McLatchey, G. P., and K.R. Reddy. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. J. Environ. Qual. 27:1268-1274.

Melillo, J. M., J. D. Aber, A. E. Linkins, A. Ricca, B. Fry, and K. J. Nadelhoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant and Soil 115:189-198.

Melillo, J. M., R. J. Naiman, J. D. Adler, and A. E. Linkins. 1984. Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bull. Mar. Sci. 35:341-356.

Miao, S. L., and W. F. DeBusk. 1999. Effects of P enrichment on structure and function of sawgrass and cattail communities in the Everglades. Ch. 11. p. 275-300. *In* K. R. Reddy, G. A. O'Connor, and C. L. Schelske (eds.) Phosphorus Biogeochemistry in Subtropical Ecosystems. Lewis Publ., New York, NY.

Miao, S. L., and F. H. Sklar. 1998. Biomass and nutrient allocation of sawgrass and cattail along a nutrient gradient in the Florida Everglades. Wetlands Ecol. Mgmt. 245-263.

Mulvaney, R. L. 1996. Nitrogen-Inorganic Forms. *In* Methods of Soil Analysis. Part 3. Chemical Methods. p.1123-1175. *In* J. M. Bingham (ed.), Soil Sci. Soc. Am., Madison, WI.

Nannipieri, P., R. F. Pedrazzini, P. G. Arcara, and C. Piovanelli. 1979. Changes in amino acids, enzyme activities, and biomasses during soil microbial growth. Soil Sci. 127:26-34.

Newman, S., H. Kumpf, J. A. Laing, and W. C. Kennedy. 2001. Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. Biogeochemistry 54:229-250.

Newman, S., and K. R. Reddy. 1992. Sediment resuspension effects on alkaline phosphatase. Hydrobiologia 245:75-86.

Newman, S., and K. R. Reddy. 1993. Alkaline phosphatase activity in the sediment-water column of a hypereutrophic lake. J. Environ. Qual. 22:832-838.

Newman, S., K. R. Reddy, W. F. DeBusk, Y. Wang, G. Shih, and M. M. Fisher. 1997. Spatial distribution of soil nutrients in a northern Everglades marsh: Water Conservation Area 1. Soil Sci. Soc. Am. J. 61:1275-1283.

Oshrain, R. L., and W. J. Wiebe. 1979. Arylsulfatase activity in salt marsh soils. Appl. Environ. Micro. 38:337-340.

Pind, A., C. Freeman, and M. A. Lock. 1994. Enzymic degradation of phenolic materials in peatlands - measurement of phenol oxidase activity. Plant and Soil 159:227-231.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29-96.

Press, M. C., J. Henderson, and J. A. Lee. 1985. Arylsulfatase activity in peat in relation to acidic deposition. Soil Biol. Biochem. 17:99-103.

Pulford, I. D., and M. A. Tabatabai. 1988. Effect of waterlogging on enzyme activities in soil. Soil Biol. Biochem. 20:215-219.

Reddy, K. R., and E. M. D'Angelo. 1994. Soil processes regulating water quality in wetlands. p.309-324. *In* W. J. Mitsch (ed.) Global Wetlands: Old World and New. Elsevier, Amsterdam.

Reddy, K. R., R. D. DeLaune, W. F. DeBusk, and M. A. Koch. 1993. Long term nutrient accumulation rates in the Everglades. Soil Sci. Soc. Am. J. 57:1147-1155.

Reddy, K. R., Y. Wang, W. F. DeBusk, M. M. Fisher, and S. Newman. 1998. Forms of soil phosphorus in selected hydrologic units of the Florida Everglades. Soil Sci. Soc. Am. J. 62:1134-1147.

Reddy, K. R., J. R. White, A. L. Wright, and T. T. Chua. 1999. Influence of phosphorus loading on microbial processes in the soil and water column of wetlands. Ch. 10. p. 249-274. *In* K. R. Reddy, G. A. O'Connor, and C. L. Schelske (eds.) Phosphorus Biogeochemistry in Subtropical Ecosystems. Lewis Publ., New York, NY.

Richardson, C. J., C. B. Craft, R. G. Qualls, J. Stevenson, P. Vaithiyanathan, M. Bush, and J. Zahina. 1997. Effects of phosphorus and hydroperiod alterations on ecosystem structure and function in the Everglades. Report submitted to Everglades Agricultural Area Environmental Protection District: Duke Wetland Center Publication #97-05, Durham, NC.

Rybczyk, J. M., G. Garson, and J. W. Day. 1996. Nutrient enrichment and decomposition in wetland ecosystems: models, analyses, and effects. Current Topics in Wetland Biogeochemistry 2:52-72.

Sarathchandra, S. U., and K. W. Perrott. 1981. Determination of phosphatase and arylsulphatase activities in soils. Soil Biol. Biochem. 13:543-545.

Saylor, G. S., M. Puziss, and M. Silver. 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Appl. Environ. Micro. 38:922-927.

Schipper, L. A., and K. R. Reddy. 1994. Methane production and emissions from four reclaimed and pristine wetlands of the southeastern United States. Soil. Sci. Soc. Am. J. 58:1270-1275.

Schipper, L. A., and K. R. Reddy. 1995. In-situ determination of detrital breakdown in a wetland soil-floodwater profile. Soil Sci. Soc. Am. J. 59:565-568.

Sinsabaugh, R. L., R. K. Antibus, and A. E. Linkins. 1991. An enzymatic approach to the analysis of microbial activity during plant litter composition. Agric. Ecosys. Environ. 34:43-54.

Sinsabaugh, R. L., M. P. Osgood, and S. Findlay. 1994. Enzymatic models for estimating decomposition rates of particulate detritus. J. N. Am. Benth. Soc. 13:160-169.

Sinsabaugh, R. L., T. Weiland, and A. E. Linkins. 1992. Enzymic and molecular analysis of microbial communities associated with lotic particulate organic matter. Freshwater Biol. 28:393-404.

Skiba, U., and M. Wainwright. 1983. Assay and properties of some sulphur enzymes in coastal sands. Plant & Soil 70:125-132.

Sparling, G. P., C. W. Feltham, J. Reynolds, A. W. West, and P. Singleton. 1990. Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content and a reassessment of the k_{EC} factor. Soil Biol. Biochem. 22:301-307.

Sparling, G. P., and A. West. 1988. Modifications to the fumigation-extraction technique to permit simultaneous extraction and estimation of soil microbial C and N. Comm. Soil Sci. Plant Anal. 19:327-334.

Sparling, G. P., and B. L. Williams. 1986. Microbial biomass in organic soils: estimation of biomass C and effect of glucose or cellulose amendments on the amounts of N and P released by fumigation. Soil Biol. Biochem. 18:507-513.

Speir, T. W., and D. J. Ross. 1978. Soil Phosphatase and Sulphatase. p.197-250. *In* R. G. Burns (ed.) Soil Enzymes. Academic Press, London.

Stams, A. J. M. 1994. Metabolic interactions between anaerobic bacteria in methanogenic environments. Ant. Leeuwenhock 66:271-294.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in Terrestrial Ecosystems. Univ. of California Press, Berkeley, CA.

Tabatabai, M. A., and J. M. Bremner. 1969. Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1:301-307.

Tabatabai, M. A., and J. M. Bremner. 1970. Arylsulfatase activity of soils. Soil Sci. Soc. Am. Proc. 34:225-229.

U. S. Environmental Protection Agency. 1993a. Determination of inorganic ions by ion chromatography. 300.0. Environ. Monit. Support Lab., Cincinnati, OH.

U. S. Environmental Protection Agency. 1993b. Methods for the determination of inorganic substances in environmental samples. 350.1. Environ. Monit. Support Lab., Cincinnati, OH.

U. S. Environmental Protection Agency. 1993c. Methods for chemical analysis of water and wastes. 365.4. Environ. Monit. Support Lab., Cincinnati, OH.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring microbial biomass C. Soil Biol. Biochem. 19:703-707.

Verhoeven, J. T. A., A. M. Kooijan, and G. van Wirdam. 1988. Mineralization of N and P along a trophic gradient in a freshwater mire. Biogeochemistry 6:31-43.

Wardle, D. A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen in soil. Biol. Rev. 67:321-358.

Webster, J. R., and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Ann. Rev. Ecol. Sys. 17:567-594.

Westermann, P. 1993. Wetland and swamp microbiology. p.215-238. *In* T. E. Ford (ed.) Aquatic Microbiology. Blackwell Scientific Publ., Cambridge, MA.

Wetzel, R. G. 1991. Extracellular enzymatic interactions: storage, redistribution, and interspecific competition. p. 6-28. *In* R. J. Chrost (ed.) Microbial Enzymes in Aquatic Environments. Springer Verlag, New York, NY.

White, J. R., and K. R. Reddy. 1999. Influence of nitrate and phosphorus loading on denitrifying enzyme activity in Everglades wetland soils. Soil Sci. Soc. Am. J. 63:1945-1954.

White, J. R., and K. R. Reddy. 2000. Influence of phosphorus loading on organic nitrogen mineralization of Everglades soils. Soil. Sci. Soc. Am. J. 64:1525-1534.

Whitton, B. A. 1991. Use of phosphatase assays with algae to assess phosphorus status of aquatic environments. p.295-310. *In* D. W. Jeffrey and B. Madden (eds.) Bioindicators and Environmental Management. Academic Press, London.

Winfrey, M. R., and J. G. Zeikus. 1977. Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. Appl. Environ. Microbiol. 33:275-281.

Wright, A. L., and K. R. Reddy. 2001a. Phosphorus loading impacts on extracellular enzyme activity in Everglades wetland soils. Soil Sci. Soc. Am. J. 65:588-595.

Wright, A. L., and K. R. Reddy. 2001b. Heterotrophic microbial activity in Northern Everglades wetland soils. Soil Sci. Soc. Am. J. (In press).

Wright, A. L., Y. Wang, and K. R. Reddy. 2001c. Influence of nutrient loading on biogeochemical indicators in Everglades soil. *In* Report to the South Florida Water Management District, West Palm Beach, FL.

Zehnder, A. J. B. 1978. Ecology of methane formation. p.349-376. *In* R. Mitchell (ed.) Water pollution microbiology, Vol. 2. John Wiley and Sons, New York, NY.

Zehnder, A. J. B., and W. Stumm. 1988. Geochemistry and biogeochemistry of anaerobic habitats. p.1-38. *In* Zehnder (ed.) Biology of Anaerobic Microorganisms. John Wiley and Sons, New York, NY.

Zibilske, L. M. 1994. Carbon Mineralization. p.835-863. *In* R. W. Weaver (ed). Methods of Soil Analysis, Part 2. Soil Sci. Soc. Am., Madison, WI.

BIOGRAPHICAL SKETCH

Alan L. Wright was born in southeast Texas. He attended Texas A&M University in College Station and received a bachelor of science degree in bioenvironmental sciences within the Department of Plant Pathology and Microbiology in 1993. Later, he earned a master of science degree in soil science from Texas A&M University within the Department of Soil and Crop Sciences in 1995. He later enrolled in the University of Florida Department of Soil and Water Science for completion of a Ph.D. program.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

K. R. Reddy, Chair
Graduate Research Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

B. L. McNeal
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D. A. Graetz
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. J. Delfino
Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D. P. Chynoweth
Professor of Agricultural and Biological Engineering

This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 2001

Dean, College of Agricultural and Life Sciences

Dean, Graduate School